SPERMATOGENESIS OF THE MYRIOPODS.

VI. AN ANALYSIS OF THE CHROMOSOME GROUP OF *Scolopenda heros*.[1]

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During the last few years a number of attempts have been made to analyze the chromosome complex of various animals. These attempts have met with such apparent success in the case of several insects, notably Orthoptera, that I have been led to attempt a similar analysis of the chromosome group in *Scolopendra heros*. Indeed, before the appearance of earlier papers upon this species such an attempt had been made, but it had met with but small success owing, as I now know, to deficiencies in the optical apparatus employed. With the facilities then at my disposal, it was impossible to secure definite clear-cut images of the chromosomes at a magnification greater than 1,500 diameters. As the chromosomes in *Scolopendra heros*, although exceedingly clear-cut and definite in outline, are considerably smaller than in some insects, a greater magnification than 1,500 diameters is necessary if the study is to be at all convincing, either to the investigator or to those reading his report. However, by the use of a Zeiss 2-mm. apochromatic objective and a number 12 compensating ocular, the source of light being a Welsbach mantle, a magnification of 2,300 diameters was obtained with no perceptible loss of definition in the image.

The material used in this study is the same which served as a basis of several previous papers[2] (Blackman :01, :03, :05), the majority of the slides having been mounted nine years, but the stain (Heidenhain's iron-alum hæmatoxylin), except where a portion of the section extends from under the cover-glass, is as perfect as when first mounted.

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[1] From the Zoölogical Laboratory, Syracuse University, Syracuse, New York.

[2] Blackman, M. W., :01, "The Spermatogenesis of the Myriapods—I., Notes on the Spermatocytes and Spermatids of *Scolopendra*," *Kans. Univ. Quart.*, Vol. 10, pp. 61–76, pl. 5–7.

Blackman, M. W., :03, "The Spermatogenesis of the Myriapods—II., On the Chromatin in the Spermatocytes of *Scolopendra heros*," BIOL. BULL., Vol. 5, pp. 187–217, 22 fig.

Blackman, M. W., :05, "The Spermatogenesis of the Myriapods—III., The Spermatogenesis of *Scolopendra heros*," *Bull. Mus. Comp. Zoöl., Harvard Coll.*, Vol. 48, No. 1., pp. 1–137, 9 pl.

Sutton,[1] :02, Robertson,[2] :08, and Nowlin,[3] :08, working upon the male cells of Orthoptera have found that the chromosomes during both the spermatogonial and spermatocyte generations may be arranged in a graded series as regards size. In the spermatogonia this series is a double one, the two chromosomes of a given size representing the similar elements derived from the two parents. These similar chromosomes unite during synapsis (Sutton, :02, *op. cit.*), and give rise to the single series characteristic of the spermatocytes. The extreme difference in the size of the chromosomes in the Orthoptera is so marked that it is noticeable at a glance (see Sutton, :02, Fig. 6) and after studying preparations of this material one cannot doubt the accuracy of their observations or deny the strength of their conclusions, that in these forms the chromosomes at any given stage bear a certain size relation to each other, and that this presents strong evidence in support of the theory of the individuality of the chromosomes.

But if we grant these conclusions with regard to the forms studied, does it necessarily follow that these conclusions should be made more general and applied to the chromosomes of all animals? What shall we say as regards the application of this test to the chromosomes of a form in which the difference in size is not so marked or in which the chromosomes all appear of nearly one size? Such is apparently the case in *Scolopendra*. At ordinary magnification there is very little difference in the size of the chromosomes as seen in a metaphase of the first spermatocyte. *Some* difference is to be detected even at a magnification as low as 1,000 diameters, but this is so slight that if size alone be used as a criterion it would seem impossible to distinguish between the chromosomes farther than to say: "This is one of the smaller ones" or "one of the larger ones."

[1] Sutton, W. S., :02, "On the Morphology of the Chromosome group in *Brachystola magna*," BIOL. BULL., Vol. 4, pp. 24–39, 11 fig.

[2] Robertson, W. R. B., :08, "The Chromosome Complex of *Syrbula admirabilis*," *Kansas Univ. Sci. Bull.*, Vol. IV., pp. 275–305, 5 plates.

[3] Nowlin, Nadine, :08, "The Chromosome Complex of *Melanoplus bivittatus* Say," *Kans. Univ. Sci. Bull.*, Vol. IV., pp. 265–271, 2 plates.

But other tests may be applied and have been applied. Baumgartner[1] (:04), made an attempt to distinguish the chromosomes in *Gryllus* by differences in form. He reaches the conclusion that in *Gryllus* certain definite shapes constantly occur and establishes the probability that there is a fixed number of each type. Davis[2] (:08), working upon various Orthoptera reaches the conclusion that "In addition to the difference in volume, the bivalent autosomes (chromosomes) show constant and characteristic differences in form. In general several more or less distinct morphological types can be distinguished, and the members of each type appear to bear a constant numerical relationship to each other."

Robertson, :08 (*op. cit.*), does not consider the shape of the chromosome of first importance in establishing its identity but considers size as the primary characteristic, while shape is secondary and to a certain extent dependent upon size or at least upon the degree of lengthening. The main criticism I wish to make regarding Robertson's conclusions on this point is that in his study of the chromosomes, he has not drawn them from the best view point to establish any characteristic difference in shape. His drawings are all or nearly all of chromosomes as seen in polar view, whereas a view at right angles to the spindle is more satisfactory in determining both the shape of the chromosomes and their relation to the mantle fibers.

In *Scolopendra*, as I have already implied, it is impossible to establish the identity of many of the chromosomes on the basis of size alone. Early in my work, however, after six or eight chromosome groups had been carefully drawn, it became evident that the chromosomes as seen in a lateral view of the metaphase of the first spermatocyte are of several distinct types as regards shape and that the size relation of the chromosomes of each type are such as to make it possible to distinguish the individual chromosomes with some degree of certainty. This, I think, will be apparent from a study of the figures of plates I. and II., although it must be borne in mind that the figures are of course much less satisfactory for this comparison than the actual chromosomes, due to the fact that many of the chromosomes do not lie at right angles to the line of vision and must, therefore, appear foreshortened in an outline drawing.

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[1] Baumgartner, W. J., :04. "Some New Evidences for the Individuality of the Chromosomes," BIOL. BULL., Vol. 8, pp. 1–23, 3 plates.

[2] Davis, H. S., :08, "Spermatogenesis in Acrididæ and Locustidæ," *Bull. Mus. Comp. Zoöl. Harvard Coll.*, Vol. LIII., pp. 59–158, 9 plates.

Before discussing the individual characteristics of the various chromosomes as seen in metaphase, it might be well to give a brief review of their history in the prophase. The spermatocyte chromosomes are seventeen in number. Of these, sixteen are bivalent elements formed by the end to end union of univalent chromosomes during the telophase of the last spermatogonial division (Blackman, '03, '05, *op. cit.*). The seventeenth element, the accessory chromosome, is univalent in character, being derived directly from a single specialized chromosome, the accessory chromosome of the spermatogonium. The growth period following synapsis is of long duration in *Scolopendra*, and during this period all of the chromosomes are grouped together to form a nucleolus-like structure to which I have given the name karyosphere. While in the karyosphere the chromosomes are so closely aggregated that their individual outlines cannot be distinguished with certainty. They, however, enter the karyosphere as distinct individuals and later arise from it as definite chromatin segments, similar in every respect except for their greater size. These facts would seem to argue for, rather than against, the individuality of the chromosomes during this stage.

In the prophase the chromosomes arising from the karyosphere are typically long, slender threads of granular chromatin, which invariably show near their middle an interruption of the chromatin—this representing the point at which the chromosomes united during synapsis. The two spermatocyte divisions always result in a longitudinal and a cross division of these bivalent elements. The longitudinal division as a rule seems to occur first, although, as we shall see later, this is not invariable, even for the ordinary chromosomes. The cross division or reduction division results in the separation of entire spermatogonical chromosomes, the division occurring at the point at which they united during synapsis. However, although the results of these divisions are the same for all of the chromosomes (with the exceptions to be noted later), the changes through which the tetrad pass in the prophase and the shapes they assume during the prophase and later during the metaphase differ to some extent. As this difference in shape is one characteristic by which we must hope to identify the various chromosomes, occasion may be taken here to describe briefly the processes by which these various forms arise.

What I shall call type A is represented in the text-figure I. The origin and evolution of this type of tetrad is described in sufficient detail in previous papers (Blackman, :03, :05, *op. cit.*); so it will not be necessary to repeat the description in detail.

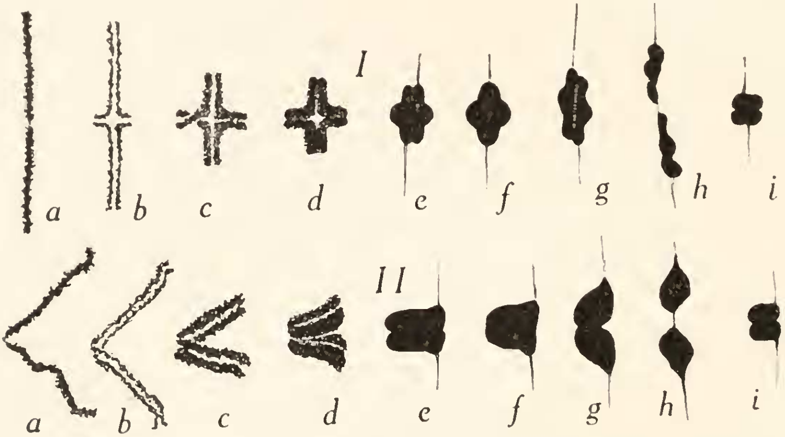


FIG. I. Semi-diagrammatic representation showing the formation and history of the cross-shaped type of tetrads; *a*, bivalent chromatin segment as it appears in the very early prophase; *b*, planes of longitudinal and transverse cleavage established; *c*, *d*, later stage in evolution of prophase tetrad; *e*, *f*, tetrads as seen in early metaphase; *g*, tetrad in act of division, showing the manner in which the component parts glide over each other; *h*, early anaphase showing distortion of halves of tetrad due to their close adhesion; *i*, daughter chromosome in metaphase of second spermatocyte.

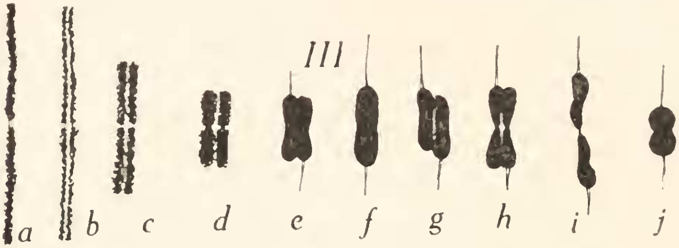
FIG. II. Corresponding stages in evolution of the double-V type of tetrad.

I wish, however, to emphasize two points. First the points at the ends of the shorter arms of the cross-like figure (Fig. I, *b*, *c*, *d*) represents the point at which union occurred during synapsis. The attachment of the mantle fibers in the metaphase is *not at this point* as it is said to be in *Syrbula* by Robertson (:08, *op. cit.*), but is at the ends of the longer arms of the cross as shown in Fig. I, *e*, *f*, *g*.

Robertson believes that the attachment of the mantle fibers coincides with the point of synaptic union of the elements and that each bivalent chromosome during its division undergoes a "change of its long axis from a longitudinal to a transverse direction." This is accomplished by a rotation of the chromatids over each other in such a manner as to result in a longitudinal division of the tetrad in the first spermatocyte. In *Scolopendra*, as I have shown in previous papers (*op. cit.*), no such complicated process occurs in division. The long axis of the tetrad in most cases remains parallel to the line of longitudinal cleavage and in the metaphase the two halves glide over each other during the act of division. As may be seen in the semi-diagrammatic drawings (Fig. I, *g*, *h*) and in several of the chromosomes of this type in the accompanying plates, the two halves of the tetrad seem to adhere rather closely and there is often considerable distortion. In Fig. I, *h*, drawn from my preparations direct it will be seen that the parts of the two daughter chromosomes remaining longest in contact are considerably lengthened and distorted apparently due to the firm adhesion of the two parts.

The second type of spermatocyte chromosome is the "double-V" tetrad described by me in a previous paper (*op. cit.*). This type usually arises from the bivalent chromosomes of the early prophase which are bent at a sharp angle at the point of synapsis. After the longitudinal cleavage of the chromatin thread has occurred the double thread becomes shorter and thicker, resulting in the double-V-shaped structure shown in Fig. II, *c*. There is at all times a very apparent interruption of the chromatin at the angle of each thread (point of synapsis), and it is at this point that the cross division occurs later. In the late prophase there is a still further condensation of the chromatin and shortening of the thread, resulting in the closer apposition of the ends of the threads farthest from the point of synapsis, resulting in a chromosome of the shape shown in Fig. II, *e*, *f*. At the time of the formation of the spindle the mantle fibers come to be attached to the distal ends (ends farthest from point of synapsis) of the tetrad in such a manner (Fig. II, *e*, *f*, *g*) that the chromosome is divided along its longitudinal axis. In this type also, the two halves of the chromosome seem to adhere closely and to divide reluctantly (Fig. II, *g*, *h*, also Figs. 6, *i*, and 17, *j*).

The chromosomes of the third type arise from thread-like structures similar to those from which type A and B arise. This thread may be either approximately straight, or it may be curved slightly in various ways, but is never bent at a sharp angle at the point of synapsis. The filament undergoes a longitudinal cleavage just as with the other types. The two resulting threads, as a usual thing, lie parallel to each other (Fig. III, *b*, *c*) but in some cases they are twisted about each other, so as to form a rope-like structure (Fig. IV, *b*, *c*). In such cases the resulting chromosome has a somewhat different shape. In this type of chromosome the tetrad resulting appears rod-shaped or double rod-shaped depending upon the angle from which the structure is viewed.



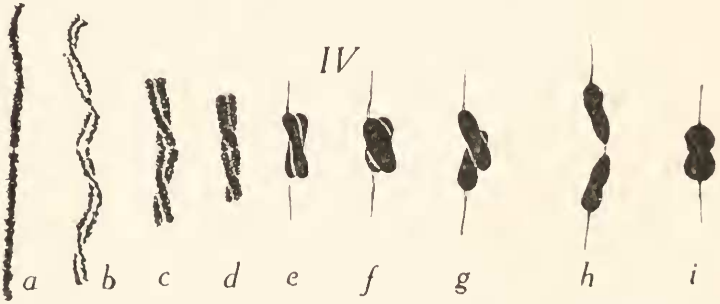


FIG. III. Evolution of the double-rod-shaped tetrads. *a*, bivalent chromatin segment; *b*, *c*, *d*, formation of tetrad; *e*, *f*, tetrads as seen in early stages of the spindle; *g*, *i*, ordinary tetrad in two stages of longitudinal division; *h*, rod-shaped tetrad apparently in act of transverse division; *j*, dyad as seen in metaphase (secondary spermatocyte).

FIG. IV. Variation of double-rod-shaped tetrad. In early prophase the double chromatin segment is often twisted as shown in *b*. The shortening of chromatin thread results in less and less twisting; so that the two parts of the metaphase chromosome merely overlie each other at an angle or are only partially wrapped about each other.

After the planes of longitudinal and cross division are established, the further changes involve merely a shortening and condensation of the entire structure. In Fig. III, *c*, *d*, two stages in this process are shown, both cleavages being very evident. Later, as the condensation continues these planes of cleavage are obscured to some extent and usually the only evidence we have of the longitudinal cleavage is a very definite notch at each end of the chromosome, while the plane of cross division is usually shown merely by a slightly lessened diameter of the tetrad near its middle (Fig. III, *d*, *e*, *f*). In some cases, however (Fig. 3, *g*, *h*), the planes of longitudinal and transverse cleavage may be seen very distinctly even in the metaphase (Fig. 4, *x*, 13, *n*, 16, *n*, etc.). In metakinesis a longitudinal division is accomplished by a gliding apart of the two halves of the tetrad (Fig. III, *g*, *i*) in a manner essentially similar to the division of the cruciform tetrads.

In a number of the cells studied there seems to be one of the chromosomes of this type, which presents quite a different appearance during the act of division. A constriction appears in this chromosome at about the middle point (the plane of transverse cleavage). This is so pronounced that there is in many cases (dependent on the stage of division) a partial or even a complete interruption of the chromatic material at this point (Figs. 3, *x*, 4, *x*, 9, *x*, 10, *x*, etc.). From a careful study of this chromosome in many cells, the conclusion seems inevitable that this one tetrad undergoes a transverse division, while the rest of the chromosomes are dividing longitudinally.

A variation of the rod-shaped tetrad is shown in text-fig. IV. Here after the longitudinal cleavage has been established, the two threads, instead of lying side by side, are twisted about each other in such a way as to form a rope-like structure. The resulting metaphase chromosome differs somewhat in appearance from others of this type, although in all essentials it is identical. As the threads shorten the twisting gradually becomes less and less pronounced (Fig. IV, *b*, *c*, *d*, *e*) until in the completed chromosomes there is only a slight twisting and usually the two parts merely overlie each other at an angle. The division accomplished by the first maturation mitosis is a longitudinal one.

Besides these three types of ordinary chromosomes all of which are bivalent there is one element which is univalent in character, being derived directly from a single spermatogonial chromosome. This is the accessory chromosome and in the metaphase can always be distinguished by its characteristic shape, and especially by the fact that it is connected by mantle fibers to only one pole of the spindle. There is usually no indication of the plane of division in the accessory chromosomes at this time, although in the prophase a longitudinal constriction is often shown.

The chromosomes of *Scolopendra heros* are seventeen in number, sixteen of which are bivalent, while one is univalent. The sixteen bivalent chromosomes undergo longitudinal and transverse divisions during the prophase and at the beginning of the metaphase are of several different types as regards shape. After studying a large number of metaphases of both the large and small (Blackman, :05) type of spermatocytes it has been found to be a rule that in each cell the chromosomes of the several types bear a definite and constant numerical relationship to each other. This fact can best be appreciated by referring to the accompanying plates. It will be seen at a glance that the cruciform tetrads which have been described above as type A are in all cases six in number. Furthermore, among the chromosomes of this typical form a definite size relation exists which makes it possible to arrange the cross-shaped chromosomes in a graded series on the basis of bulk. To be sure, the difference in size is not so striking as that existing between the largest and smallest chromosomes in some insect material, but I believe is as great as the difference in size between adjacent chromosomes of the graded series in insects. It is perhaps unnecessary to explain that the *actual* size difference is in many cases greater than appears in the camera lucida drawings, owing to the fact that some of the chromosomes are foreshortened on account of the angle from which they are viewed. For this reason, the drawings are much less convincing than the preparations.

The shape of the chromosomes, aside from showing an apparent modification due to the angle of vision, actually does vary considerably but each of the group of six chromosomes in question are always reducible to the cruciform tetrad described as type A. The variations in shape have to do only with the degree to which the short arm of the cross is drawn out and to apparent differences incident to the angle of vision. When the short arm of the cross is drawn out but little the tetrad approaches the rod-shaped chromosomes of type C. Then, too, the cruciform tetrads vary in shape in different stages of the metaphase. The limbs of the cross are more likely to be of nearly equal size in the early spindle stages than when division has actually begun. As the chromosomes even in the same equatorial plate, are not all in exactly the same stage of division at the same time this factor should receive consideration.

An attempt was made to learn whether any of the chromosomes constantly either lag behind or precede the others in division. It was found that chromosome A, the largest of the cross-shaped tetrads, shows a tendency to lag somewhat behind the others of this type. In many of the chromosome groups this is very evident. In some cells it is not yet oriented in the characteristic manner in the equatorial plate at a time when some of the others have begun to show the characteristic change in shape incident to the gliding apart of their component elements. This can be seen by comparing chromosome A with other chromosomes of similar type in Figs. 1, 3, 4, etc. Then, too, this chromosome often presents a less clear-cut outline than do the others, approaching the granular condition characteristic of the prophase.

Of the chromosomes of type B there are five present in the metaphase of *Scolopendra heros*. This is the type of chromosome which shows the characteristic double-V shape in the prophase. The difference in shape between the tetrads of this type and the cross-shaped elements is usually quite striking. Even more characteristic of this type is the attachment of the mantle fibers and the orientation of the chromosomes in the equatorial plate. As seen in side view, the chromosome is more or less rectangular in shape, but one end is usually wider than the other and to the angles of this end the mantle fibers are attached. The chromosome usually lies with this end toward the center of the spindle, while the free end (that to which the mantle fibers do not attach) extends outward. This free end is often notched and this notch indicates the plane of longitudinal division. In end view (Figs. 1, *I*, 6, *J*, etc.) the appearance is not so characteristic in the drawings, although in the preparations there is no difficulty in recognizing the true shape of the chromosome, the apparent difference in shape being due to the view-point from which it is seen.

The five chromosomes of type B form a graded series as regards size, just as with those of type A. The largest one is very perceptibly greater than the smallest, and the intermediate ones differ in size to such an extent that there is usually little difficulty in assigning them to their proper place in the series. No individual of this type shows any constant precocity or tardiness in division, although in some cells one or more of them are farther along than the others (Figs. 6, *I*, 17, *J*).

The rod-shaped tetrads (type C) are five in number in *Scolopendra heros*. They show the same constancy in size relation as do the other types, and may be readily arranged in a graded series· Usually one or more of this type of chromosome show the component parts overlapping each other at an angle or partially wrapped around each other, indicating that they arise from the twisted threads often seen in the prophase and already described. However, these are not constant in occurrence and this condition seems to depend largely upon chance.

A fact which has proved rather puzzling was brought out by a careful study of the various chromosomes of this type. While it cannot be doubted that four of the rod-shaped chromosomes divide longitudinally in the first spermatocyte division, the fifth tetrad of this shape apparently divides transversely. In all cases in which this element is well advanced in the metaphase there is a very evident constriction at its middle point, and in some cases this amounts to a nearly complete interruption of the chromatic material. This is especially evident in Figs. 3, 4, 10, chromosome *N*. Indeed, it seems hardly possible to escape the conclusion that at the same time the other fifteen bivalent chromosomes are undergoing longitudinal division, this one element is divided reductionally.

This, however, is no less to be expected than is the behavior of the accessory chromosome in this same division. It differs from the other chromosomes in being univalent (*i. e.*, it has no synaptic mate), while the rest are bivalent. After the formation of the spindle it lies among the other chromosomes and is scarcely distinguishable from the rod-shaped ones aside from the fact that it is connected by mantle fibers to only one pole of the spindle. It is not divided by the first spermatocyte division but passes to one pole entire. Thus the result is in a sense similar to a reductional division, the two cells differing as regards the distribution of this element. It has no synaptic mate and is, therefore, distributed to only one of the resulting cells (Figs. 19, 20).

It is evident that the unequal distribution of the accessory chromosomes produces two sorts of secondary spermatocytes—one half having only the sixteen ordinary chromosomes and one half having the accessory chromosome in addition. By the second spermatocyte division, the accessory chromosome is divided and as it occurs in but half of the second spermatocytes it is distributed to only half of the spermatids, thus giving rise to a dimorphism among the spermatids and spermatozoa. The significance of this dimorphism has been discussed by a number of investigators—McClung[1] (:02), Wilson[2] (:06), Stevens[3] (:08, :08*a*, :09) Boring[4] (:07) and others—and, as I have nothing new in the way of observations to offer it would appear hardly profitable to consider the subject in detail. I believe, however, that when the chromosomes of the female germ cells of *Scolopendra* are studied it will be found that these are thirty-four in number in the ovogonia, and that the following fertilization formulæ of Wilson (:06, *op. cit.*) will hold good for this species:

$$\text{Egg } \frac{X}{2} + \text{Spermatozoon } \frac{X}{2} \left(\begin{matrix}\text{including}\\ \text{accessory}\end{matrix}\right) = X \text{ (female).}$$

$$\text{Egg } \frac{X}{2} + \text{Spermatozoon } \frac{X}{2} - 1 \left(\begin{matrix}\text{accessory}\\ \text{lacking}\end{matrix}\right) = X - 1 \text{ (male).}$$

[1] McClung, C. E., :02, "The Accessory Chromosome—Sex Determinant?" BIOL. BULL., Vol. 3, pp. 43–84.

[2] Wilson, E. B., :06, "Studies on Chromosomes—III., The Sexual Differences of the Chromosome Group in Hemiptera, with some Considerations on the Determination and Inheritance of Sex," *Journ. Exp. Zoöl.*, Vol. III., pp. 1–40, 6 fig.

[3] Stevens, N. M., :08, "A Study of the Germ Cells of Certain Diptera with Reference to the Heterochromosomes and the Phenomena of Synapsis," *Journ. Exper. Zoöl.*, Vol. V., pp. 359–374, 4 plates.

Stevens, N. M., :08*a*, "The Chromosomes in *Diabrotica vittata*, etc.," *Journ. Exper. Zoöl.*, Vol. V., pp. 453–469, 3 plates.

Stevens, N. M., :09, "Further studies on the Chromosomes of Coleoptera," *Journ. Exper. Zoöl.*, Vol. VI., pp. 101–113. 4 plates.

[4] Boring, Alice M., :07, "A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ," *Journ. Exp. Zoöl.*, Vol. IV., pp. 469–512, 9 plates.

In *Scolopendra* (Blackman, :05, *op. cit.*) there are two distinct types of spermatocytes readily divisible on the basis of size. Those characterized by the larger size are about twice the average diameter of the smaller ones and vary from them in behavior in the two maturation divisions. But this variation in behavior concerns the achromatic structures of the cell and seems to be due to the much greater amount of cytoplasmic and archoplasmic material present in the larger cells. It is extremely interesting to note that as regards the behavior of the chromosomes these two sizes of spermatocytes are essentially identical. Indeed, the chromosome groups of the small cells differ in no respect from those of the large type. The elements are no smaller than in many of the large cells and present the same constancy in form and the same size relations characteristic of the large type of cells. Figs. 16, 17, 18 represent the chromosome groups of the small type of spermatocytes. Many more were carefully studied and drawn and all show the same characteristic shapes and size relations typical of the spermatocyte chromosomes. In fact, the only reason more of these were not used is that they are not so desirable for study owing to the difficulty in drawing them due to their closer crowding in the metaphase.

The shape of the daughter chromosomes as they move apart to the poles in the anaphase of the first spermatocyte mitosis is quite characteristically different for the different types of chromosomes (Figs. I, II, IV, *h*, III, *i*). Those resulting from the division of cross-shaped tetrads have the three lobed appearance shown in Fig. I, *h*. The daughter chromosomes resulting from the division of the double-V-shaped tetrads have the shape shown in Fig. II, *h*, and are essentially single-V-shaped chromosomes, as is shown at a later stage. Those resulting from the division of the double-rod tetrads, as they move toward the poles, have the form of single rods, slightly constricted near the middle. I have been unable to identify positively the division products of the tetrad which undergoes its reduction division in the first mitosis, but in several anaphases six of the daughter chromosomes of each group are V-shaped, and it is probable that the sixth one of this shape is the chromosome in question. This is rendered more certain by the observation that in these cells there are but four of the rod-shaped chromosomes exclusive of the accessory, which, of course, is present in but one of the chromosome groups.

The chromosome groups, as seen in the metaphase of the two cells derived from one primary spermatocyte are shown in Plate II., Figs. 19, 20. The most striking fact to be observed is the absence of the accessory chromosome in one of the cells. Even a superficial examination, however, shows that in shape and relation to the mantle fibers the chromosomes are of several different types, and that these characteristics coincide with what would be expected from a study of their earlier history. The chromosomes derived from the cross-shaped tetrads have altered their shape considerably since last seen in the anaphase of the first maturation division. They are much shorter and thicker and are now bilobed bodies—the constriction between the lobes representing the plane of transverse cleavage. The attachment of the mantle fibers seems to be at no particular point but may be at any part of each flattened end of the dyad.

The structural peculiarities of the chromosomes derived from the double-V-shaped tetrads are much more characteristic. In shape the dyads of this type resemble those just described to some extent, but, except in size, bear a more striking resemblance to the double-V-shaped chromosomes from which they are derived. One end, usually the one nearest the center of the equatorial plate, is broader than the other, and the entire structure very evidently corresponds to the single-V-shaped chromosomes of the first spermatocyte anaphase. The mantle fibers are always attached to the broader end of the dyad, this fact being even more characteristic than the shape. The appearance of these chromosomes while in the act of division might lead one to believe that the resulting division is a longitudinal one, but such a conclusion would ignore entirely the previous history of this type of chromosomes during the prophase and metaphase of the first maturation division.

Chromosomes L, M, O and P, in the second spermatocyte (Figs. 19, 20) are the product of the longitudinal division of the rod-shaped tetrads. They are dumbbell-shaped dyads with a mantle fiber attached to each end. The constriction at the middle of each represents the plane of transverse division. Chromosome X shows a considerably different shape, corresponding to its different history. It is shorter and in one plane broader than the other dyads derived from the double-rod-shaped tetrads of the first spermatocyte. It has been already shown that chromosome X of the first spermatocyte probably undergoes a transverse division while the other tetrads are dividing longitudinally, and we would, therefore, expect the products of this division to present a different appearance from the other dyads. As a matter of fact, it is of quite a different shape from the others derived from the double-rod tetrads.

The differences in size between the various chromosomes of the different types is, of course, only half as great in the second spermatocyte, as it is in the first spermatocyte, and therefore there is not such certainty in identifying the various individuals of the different types. But the same size ratio seems to exist and the chromosomes of the different types can readily be arranged in a graded series as regards size, just as in the first spermatocyte.

It has been shown by this study that the chromosomes of *Scolopendra heros* cannot be considered as ephemeral structures, which have one appearance in one cell and present an entirely different form in another cell of similar history. Any study except a very superficial one must lead to an entirely different conclusion. By a study of many hundreds of cells in various stages of mitosis it has been found that the number of chromosomes in the primary spermatocytes is absolutely constant and invariable. Furthermore, these chromosomes show other characteristics, which speak very strongly for their individuality. The ordinary chromosomes are divisible into three types on the basis of the shape they assume in the prophase and metaphase of the first maturation division, and in their relation to the mantle fibers of the spindle. The individuals of each type of structure are invariably of the same number and in all favorable cases each chromosome of a given type is distinguishable from the others of a similar shape by a difference in size.

In addition, several of the chromosomes possess certain individual peculiarities aside from shape and size, which serve further to characterize them. One of the cross-shaped tetrads is tardy in orienting itself in the plane of the spindle. Another chromosome, one of the rod-shaped ones, shows a much more striking and fundamental peculiarity, in that it differs from all of the rest of the bivalent chromosomes in the plane of its divisions in the first and second spermatocytic mitoses. The accessory chromosome shows still more striking peculiarities, differing from the others in its origin, valence, behavior in the prophase, relation to the mantle fibers of the spindle and in its distribution to but one half of the resulting cells.

All of the facts enumerated above offer evidence which seems conclusive that the chromosomes of *Scolopendra heros*, during the spermatocyte stages at least, must be considered as distinct entities, each one possessing certain well defined peculiarities which are as characteristic for any given chromosome of the spermatocytes as are the peculiarities of a species of animals. I believe that eventually in many animals it will be possible to make this statement still broader and to demonstrate the continuity of the individual elements from cell generation to cell generation. We will, then, be able to say that, while in different cell generations or different conditions of cell activity, the appearance and behavior of any given chromosome may be quite different, just as is true of many animals in different stages of their existence, yet in a similar cell generation any particular chromosome will present the same appearance and will behave in the same manner. I believe that the condition described above is true in *Scolopendra heros* but several facts conspire to make it impossible of physical demonstration.

These difficulties are mechanical difficulties and have to do with the small size of the chromosomes in the spermatogonial stages and the close aggregation of these elements in the karyosphere of the growth period. The difficulties due to the small size of the chromosomes in the spermatogonial stages appears insurmountable, and the only evidences of individuality which they present have to do with their absolute constancy in number, and with the very characteristic behavior of the accessory—it being the only element which can be identified at all stages. We might reason from this that because one of the elements displays unmistakable individuality all of the chromosomes possess individuality. This argument has been made in other cases and, while the continuity of the accessory chromosome *does* offer valuable evidence in support of the individual continuity of the chromosomes in general, it cannot be said to establish the truth of the general theory.

The difficulty of establishing the individuality of the chromosomes during the growth period would seem fully as great as during the spermatogonial period. During all the stages in which the karyosphere exists the chromosomes are so densely aggregated that it is impossible to distinguish the separate elements. But even at this time it is possible in favorable cases to distinguish the accessory chromosomes and to discern the outlines of some of the other elements. Furthermore, as I have shown in previous papers (Blackman, :05, *op. cit.*) the chromosomes enter the karyosphere as distinct bivalent elements, and at the end of the growth period arise from it as distinct chromatic segments of the same number and character as in the earlier stage.

The chromatin segments entering the karyosphere are bivalent threads formed by the union and subsequent diffusion of two spermatogonial chromosomes. The point of union of synapsis shows very plainly as a distinct interruption of the chromatin granules near the middle of the segment, the interval being bridged by linin fibers. In favorable sections of the karyosphere (*i. e.*, those in which the stain has been sufficiently extracted) it is seen that this body is made up of a number of chromatin segments closely massed about the accessory. The chromosomes on leaving the karyosphere are of the same structure as when they entered, are of the same number and in appearance differ from those of an earlier period in size only. In fact, the larger spermatocyte chromosomes possess nearly as great a bulk as the entire chromosome group of the spermatogonium, this immense increase in size being accompanied by a growth of other parts of the cell, which is proportionally even greater.

It would appear then, that during certain stages of the spermatogenesis of *Scolopendra* it is possible to demonstrate absolutely that each chromosome is a distinct unit characterized by certain definite and constant peculiarities and that the continuity of each element can be traced from the early prophase of the first spermatocyte to the anaphase of the second maturation division. In other words, it is evident that during this very important period of their history the chromosomes show complete individuality. In other stages, namely, in the spermatogonia and during the growth period, it cannot be claimed that the continuity of the chromosomes is actually demonstrated in *Scolopendra*, although evidence strongly supporting such a view undoubtedly exists.

SUMMARY.

The chromosome group of the primary spermatocytes of *Scolopendra heros* is made up of sixteen bivalent chromosomes (tetrads) and one univalent chromosome (dyad), the accessory chromosome.

The chromosomes show such constancy in shape in the prophase and metaphase of the primary spermatocytes, and in their relation to the mantle fibers of the first maturation spindle, that they seem naturally to group themselves under four distinct types. These may be designated respectively, as the cross-shaped tetrads, the double-V-shaped tetrads, the rod-shaped tetrads, and a single-rod-shaped dyad.

The cross-shaped tetrads are six in number and may be arranged in a graded series as regards size, the difference in bulk being sufficiently great to allow the individual chromosomes of this type to be distinguished. One of the chromosomes of this type (the largest one) can furthermore often be identified by its tendency to lag behind the others during the early metaphase.

Five of the tetrads are of the double-V shape. The individuals of this type also may be distinguished by differences in bulk.

The rod-shaped tetrads are present to the number of five. These show constant size relations and may readily be arranged in a graded series as regards magnitude. One of the tetrads of this type differs from the others in the form it assumes during actual division. It seems to divide transversely, while the others are dividing longitudinally.

The accessory chromosome is univalent and passes to one of the secondary spermatocytes without division. During the metaphase it is connected by mantle fibers to only one pole of the spindle.

As a result of the first spermatocyte mitosis fifteen of the tetrads are divided longitudinally (equationally), while the one remaining tetrad divides transversely (reductionally). The failure of the accessory chromosome to divide is, also, in effect a reductional division.

During the later stages of the first maturation division and during the metaphase of the second spermatocyte, it is possible to distinguish the daughter chromosomes derived from the several types of tetrads, by their shape and their relations to the mantle fibers. The individuals of the various types show the same size ratio as exists between the chromosomes of the first spermatocyte, although, of course, the actual difference in bulk is but half as great.

The above results seem to establish as a fact, or at least as a very strong probability, that the chromosomes of *Scolopendra heros* are distinct and definite individuals, which, under similar circumstances, *i. e.*, in the same cell generation, show a remarkable constancy in form, relative size, and in their attachment to the mantle fibers. This constancy of form, size and behavior, affords a strong argument in favor of the theory of the individuality of the chromosomes in this species in particular and adds support to the evidence derived from the study of other forms, to the general application of the theory.

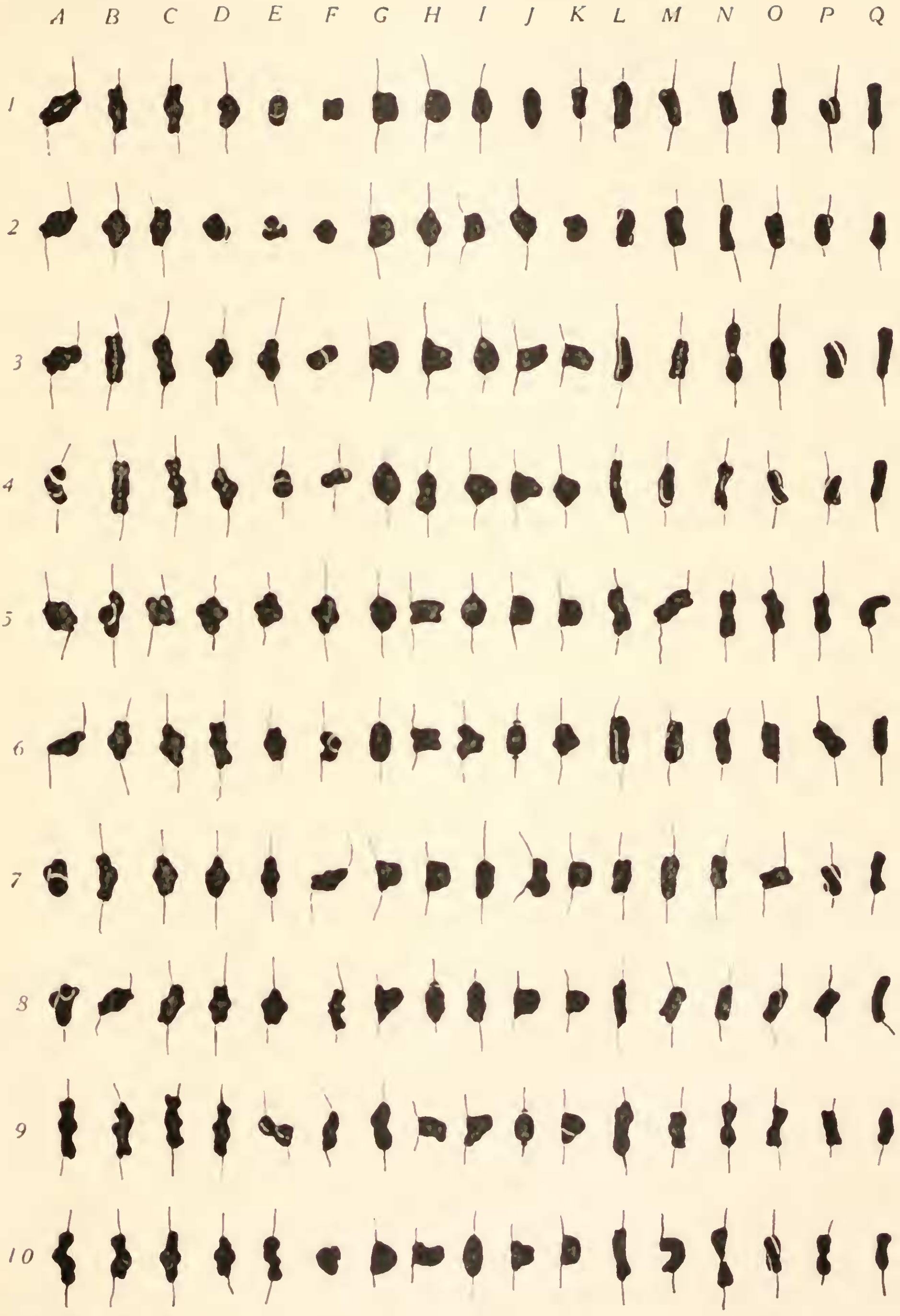
Laboratory of Zoölogy,
Syracuse University,
April 11, 1910.

Explanation of Plate I.

All drawings were made by the author with the aid of a camera lucida. The optical equipment consisted of a Zeiss apochromatic objective of 2 mm. focus and a number 12 compensating ocular, the source of the light being a Welsbach mantle The original magnification was 2,300 diameters and the drawings were reduced one fifth in reproduction, making the final magnification 1,840 diameters.

The seventeen chromosomes arranged in each horizontal row represent the chromosomes of a single cell, as seen in a side view of the spindle in the metaphase. They are arranged as follows: the six which show the characteristic cruciform shape comprise the first six of each row and are lettered A, B, C, D, E and F. Those showing the double-V shape—five in number—are lettered G, H, I, J and K. Those corresponding to the double-rod type of structure are lettered L, M, N, O and P. The seventeenth and last chromosome in each row is the accessory and is distinguished by the letter Q. The individuals of each type of structure are further arranged in a graded series as regards size, the largest first, etc.

Figs. 1–15 represent the chromosome groups in the metaphase of the large type of first spermatocytes.



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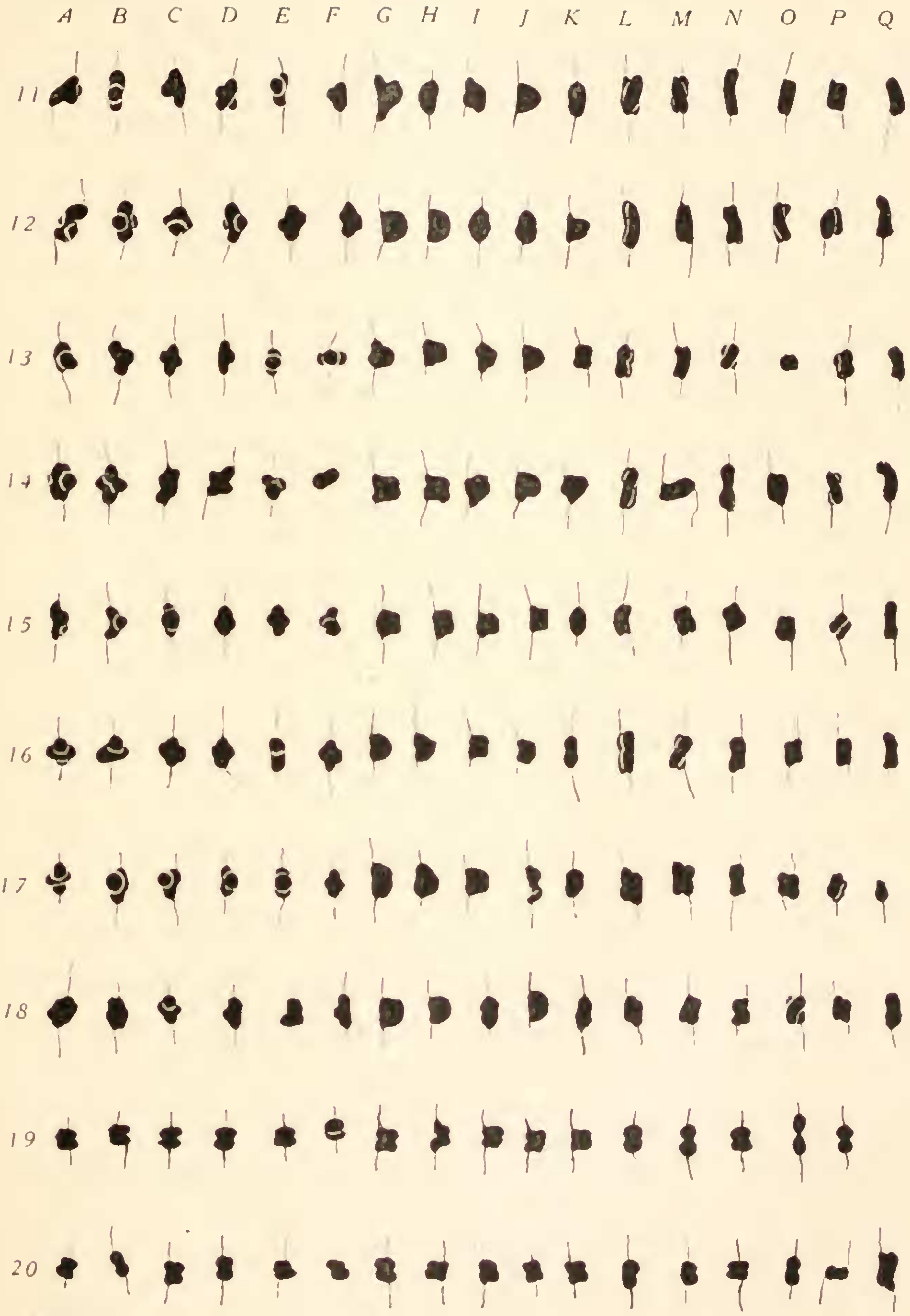
Explanation of Plate II.

Figs. 16–18 represent the chromosomes of the small type of spermatocyte in a similar stage.

Figs. 19 and 20 represent the chromosomes of the two second spermatocytes, derived from one primary spermatocyte. The individual chromosomes are arranged so as to correspond in position to the parent chromosomes as seen in the other figures. As will be seen, in one cell the accessory chromosome is not present, it being distributed to only half of the secondary spermatocytes.

PLATE II.



M. W. BLACKMAN

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BIOLOGICAL BULLETIN

MANGANESE OF THE LAMELLIBRANCHS.

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In 1892 Griffiths published an account of the finding of manganese in the blood of the lamellibranch, *Pinna squamosa*.[1] So far as I am aware this result has never been confirmed nor until recently has an examination of other molluscs led to an extension of this isolated fact. To the student of comparative physiology such a finding must be of considerable interest, adding one more respiratory mechanism to the list of five or six with which we are familiar. At the same time it is highly improbable that *Pinna squamosa* is the only mollusc utilizing manganese in a respiratory compound: we should expect to find the element in other forms more or less closely related to it. It is a matter of common observation that the respiratory proteins of the more highly organized animals fall into a few general types, such as hæmoglobin or hæmocyanin, and that while individual bloods may show subtle biological differences within one of these groups, there is never any difficulty in determining chemically whether a blood pigment is a hæmocyanin, a hæmoglobin, or some other typical complex. The effective respiratory mechanisms are thus quite limited, so that we do not expect to find a single member of a family possessed of a blood protein totally unlike the other members of that family.

For this reason we have extended the investigation of this point somewhat with a view to determining what other lamellibranchs are provided with a respiratory mechanism similar to that of *Pinna squamosa*. The most notable group which we have thus far found to utilize the element manganese is the Unionidæ, the common fresh-water mussels. Since 1906 when the element was first noticed in the specimens obtained from the Madison Lakes of Wisconsin,[1] we have examined many hundreds of specimens from the Mississippi basin, St. Lawrence and Atlantic coast drainage. In not a single specimen has the element been wanting or small in amount. It is obvious that a single individual which failed to show manganese, or contained only a trace of it would be sufficient to cast grave doubts upon the normality of the element and lead one to ascribe an adventitious character to it. But in every case manganese has been abundant. The reactions for its identification are fortunately brilliant and decisive and at the same time indicate very well the relative amount of the element. The quantitative determinations show that the metal occurs in quite uniform amounts in the various specimens examined.

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[1] Griffiths, *Compt. rend. de l'Acad. des Sci.*, CXIV., p. 840.

To summarize briefly the results: Some twenty-four analyses were made quantitatively upon material from about Madison. Some of these analyses were made upon single specimens of *Anadonta* or *Unio*, more were made upon a sample taken from the dried and pulverized tissues from a large number of specimens secured at one time from a given locality. Many of these analyses therefore represent the average of fifty or a hundred individuals. The average of the twenty-four analyses shows 21.8 per cent. of ash in the tissues, 4.52 per cent. manganese present in the ash and 0.95 per cent. manganese in the tissues. Mussels from the Wisconsin River averaged about 14.5 per cent. ash, 2.4 per cent. manganese in the ash and 0.35 per cent. in the tissues. From the Temagami Reserve of Ontario mussels averaged 15.4 per cent. ash, 3.1 per cent. manganese in the ash and 0.45 per cent. in the tissues. Specimens obtained from a great number of localities in Michigan, Illinois, Wisconsin, Indiana and Iowa average about 17.0 per cent. ash, 3.4 per cent. manganese in the ash and 0.60 per cent. in the dry tissue. A number of normal, average sized specimens from Lake Mendota were dissected into their more prominent tissues or organs. Analyses of these fractions gave the following results:

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[1] Bradley, *Jr. Biological Chem.*, III., 151, 1907.

Tissue.	Per Cent. Ash.	Per Cent. Mn in Ash.	Per Cent. Mn in Tissue.
Muscle	6.0	4.87	0.293
Stomach; fibrous part	14.5	5.73	0.831
Stomach; non-fibrous part	32.0	4.66	1.492
Nephridial organs	27.0	5.31	1.434
Gills	33.5	4.89	1.638
Mantle	48.0	5.12	2.457
Liver	39.0	5.85	2.107
Eggs	37.0	2.024	0.749

Perhaps the most interesting result of these analyses was the presence of the element in the eggs and embryos, showing clearly that manganese is not an adventitious element picked up by the adult and held in the tissues from inability to excrete it—as, for example, iron compounds may be in the mammalian spleen after certain diseases involving great destruction of red corpuscles.

Another interesting point brought out in the above table is the exceedingly high mineral content of such a tissue as the mantle. Its ash content of 48.0 per cent. puts it in a class with mammaliam osseous tissue, though unlike the latter the mantle is soft and pliable. This is a type of tissue resembling no vertebrate organ or tissue of which we are aware. It seems probable that its function as a gland secreting the shell must have some connection with the high mineral content.

Having established as we believe the normality of the element manganese in the tissues of the Unionidæ, the question as to its origin naturally presents itself. It is hardly to be supposed that an animal of so great complexity as a lamellibranch would actually concentrate the element from its highly dilute solutions in lakes and streams. Such concentration is usually performed by lower forms of life which are then obtained as food. In the case of the Unionidæ the food origin of manganese is much more clearly apparent than is the food origin of copper in many of the hæmocyanin-bearing animals. The waters in which the mussels are found have invariably contained the brown masses of the manganiferous crenothrix mixed with diatoms, and other planeton forms which very probably contain manganese also. And this brown slime seems to be the normal food of these mussels so far as our observations extend. In Ontario there are many lakes set in clean rocky basins, and fed by streams which leave little or no manganese stain on the rocks, and which appear to be free from the masses of crenothrix. In such lakes we have been unable to find mussels. In other lakes in the same region where seepage through glacial drift was apparent, or where the tributary streams flowed through such drift, discoloration of the stones, evidence of the presence of crenothrix, and the presence of the mussels seemed always to go together. For example in the Temagami Reserve, Lake Temagami itself is characterized by its clear water, and its clean rock basin. In certain parts occur limited areas of drift—sand and gravel—which are of insignificant amount. But though the bottom afforded, where the lake washed such drift areas, looked promising no mussels were found and the sandy stretches were apparently free from crenothrix. To the north of Lake Temagami are several lakes which lie in basins of glacial drift. In Sucker Gut, for example, sand and gravel beaches are abundant, the tributary stream flows through many miles of drift and carries enough manganese and iron in solution to stain its stones and pebbles strongly brown and black. The sand itself is stained with iron, and the brown slimy masses of crenothrix are abundant. In this lake and its tributary stream we found enormous numbers of small mussels wedged in thickly between pebbles or projecting from the sandy bottom. The many obvious examples in this region of the simultaneous presence of manganese, crenothrix and mussels, or of the absence of all three is probably more than a mere coincidence. We believe that more careful examination would show that the mussels require such manganiferous food as crenothrix and that they cannot live in waters where such food does not thrive.

In growing the mussels in aquaria the specimens always carry enough of the manganiferous organisms clinging to them so that in a few days an abundant development of the bacteria results. In this way several hundred grams of the dry organisms have been obtained for analysis. Such specimens are mixtures of a great variety of organisms and thus show large differences in chemical content. The ash content of such plancton crops vary from 24 to 76 per cent. of the dry weight; the manganese from 0.13 to 1.84 per cent. of the dry weight. It has thus been possible to obtain, through the agency of these organisms, several grams of manganese from running water which contained about 0.0000066 per cent. of that element. The concentrating efficiency of these lower forms is therefore of a high order.

In secreting the shell, the Unionidæ deposit salts of manganese as well as of calcium and magnesium. The nacer of the shells, carefully freed from contaminating material, always gives a strong reaction for manganese; its presence in the shell is as characteristic as its presence in any of the tissues. It was thought therefore that an examination of fossil shells of the Unionidæ would be of interest in determining whether the manganese is of comparatively recent occurrence in these animals or whether it is a metabolic characteristic of long standing. So far we have had the opportunity to examine but one well preserved fossil shell. This was a specimen obtained through the courtesy of Dr. George Wagner who published a description of it in *Nautilus*, Vol. 18. The nacer of this shell was perfectly preserved, retaining its luster, though friable and crumbling to a powder easily. The fossil nacer gave 0.085 per cent. of manganese, while fresh shells of the present period frequently contain as much as 0.148 per cent. Thus it can be definitely asserted that the Unionidæ in pre-Pliocene times were using the element manganese as we find them today. It seems probable that the marine ancestors of the Unionidæ were themselves manganiferous. The fact that at least one marine lamellibranch is known makes such an assumption the more plausible.

To determine whether other marine lamellibranchs utilize manganese in this same way, an examination of the common forms along the coast of southern Massachusetts was made at the Woods Hole laboratory. In several forms the elements could usually be detected as a trace, but in such cases no import can be attached to its presence except as indicating that there is some marine low form of life serving as food for lamellibranchs, which also carries manganese. In *Pecten* the manganese was variable, sometimes large in amount, at others very small. It was frequently found abundant in the stomach contents. In *Modiola modiolus* the element was present in every specimen examined, and it seemed to be rather uniform in amount. It was present in every tissue, and in the nephridial organs it was really abundant. In most of the tissues it was not at all comparable to the amount present in the Unionidæ, approximately 0.1 per cent. or less of the dry material. It will be remembered that the nephridial organs of *Modiola modiolus* are usually pigmented a dark brown—in all of some fifty specimens examined by us this brown pigmentation was prominent. It is possible that this lamellibranch deposits manganese obtained with its food in the nephridia in an attempt to excrete the element; that it is in this case adventitious and analogous to deposits of iron-containing pigment in mammalian tissues as the result of pathological conditions. It is interesting to note however that this marine mussel which stands morphologically fairly close to the Unionidæ, should appear to utilize the element so characteristic of the latter family.

It is our belief that other lamellibranchs will be found which, like *Pinna squamosa*, the Unionidæ and perhaps *Modiola modiolus*, utilize the element manganese in their metabolic processes. Such a chemical relationship may be useful in suggesting the lines of the evolutionary process which has led to the development of the present forms. It is our expectation to continue this line of investigation as opportunity permits.

THE SPERMATOGENESIS OF EUCHROMA GIGANTEA.

M. LOUISE NICHOLS.

The largest of the buprestid beetles, *Euchroma gigantea*, is native to Central and South America and is commonly found sunning itself on the trunks of trees. In such situations the beetles are not difficult to capture, as their movements are rather sluggish until they become thoroughly alarmed. The specimens from which the present study was made were taken at Culebra, Panama, in the month of August, at which time some of the beetles were mating, the male apparently attracting the female by a clicking sound produced by the elytra.

Upon sectioning the testes, I was surprised to find a complete series of stages from the spermatogonia to the mature spermatozoa, the younger stages not being confined to the larvæ or pupæ, as is frequently the case in insects. The testes were fixed in Gilson's mercuro-acetic-nitric solution or in Fleming's strong solution and stained with iron-hæmatoxylin or with saffranin and malachite green.

In the development of the germ cells of insects, as is well known through the results of the researches of Montgomery, Wilson, Stevens and others, there are present chromosomes which have been called heterochromosomes or idiochromosomes. Wilson (1909) has shown for the Hemiptera that in certain forms the idiochromosomes are equally well developed in both sexes, in others the male possesses one well developed, the other reduced in size, while in still others one is entirely lacking in the male. Stevens (1906) found somewhat similar conditions in the Coleoptera. Thus, the Elateridæ and Lampyridæ possess only the odd chromosomes, while the families Chrysomelidæ, Coccinelidæ, Scarabidæ, Silphidæ and Buprestidæ show one of the idiochromosomes reduced in size. In Carabidæ some members have an unequal pair of heterochromosomes, others an odd chromosome. *Euchroma gigantea*, as a member of the family Buprestidæ, belongs in the second of these groups (Figs. 21–22).

Besides the idiochromosomes, Wilson discovered in the Hemiptera a pair of chromosomes equal in size but noticeably smaller than the others, which he designated as *m*-chromosomes. According to the researches of Stevens these are occasionally present in the Coleoptera, *i. e.*, in *Trirhabda virgata* and *T. canadense* and in an unidentified buprestid. They likewise are represented in *Euchroma* (Figs. 19, 23, 24). In addition there are, in the spermatocytes, eleven chromosomes of more nearly equal size, making the total reduced number thirteen.

In most forms heretofore studied, the idiochromosomes are evident not only at the time of mitosis but also in the resting stage and prophases, for while the other chromosomes become resolved into the nuclear network, the idiochromosomes remain compact. It is in the manner of formation of the chromosomes during the prophases of the first maturation division and in the fact that neither at that time nor in the previous stages are the idiochromosomes distinctly different in behavior from the other chromosomes that the chief interest of the spermatogenesis of this beetle lies.

The nuclear network of the last generation of spermatogonia is of delicate texture. Chromatin masses occur at intervals, at first few in number and without constancy of position or shape (Fig. 5). The masses gradually become more distinct and form elongated threads near the center of the nucleus (Figs. 6, 7). The network breaks away from the nuclear wall and the synapsis is inaugurated (Figs. 8, 9). During this time there is no evidence of the idiochromosomes being isolated from the synaptic threads or failing to take part in their formation, nor, in the resting spermatocyte, do the idiochromosomes differ from the others. Stevens (1906) has reported a somewhat similar condition in the beetle *Tenebrio molitor*.

The nuclear network of the resting spermatocyte is more clearly defined than that of the spermatogonia and bears chromatin masses distributed with a fair degree of regularity (Fig. 10). This condition, however, does not continue. Instead of the usual spireme formation, the chromatin granules commence to migrate towards a specialized area within the nucleus (Figs. 11–14). The final result of this process is the formation of a dense mass of chromatin in one part of the nucleus, the remainder of the nucleus being occupied by a fine network. It sometimes happens that more than one of these areas of aggregation develops (Figs. 15–18).

The outlines of the separate chromosomes may be seen, although they lie very close together. The nuclear network next begins to show a parallel arrangement of threads preparatory to the formation of the spindle (Figs. 17–18).

As is the case with other members of this order, the small heterochromosome is separated from the larger by the first division (Fig. 21). In mitosis the *m*-chromosomes tend to divide somewhat later than the others (Figs. 23–25).

After the second division the spindle fibers persist in the cytoplasm. They gradually cease to run parallel to each other, become more or less interlaced, and finally are arranged in spiral form (Figs. 26–28). Later they are converted into the tail of the spermatozoön (Fig. 32).

The chromatin of the spermatid at first condenses in large measure at the side of the nucleus nearest the spindle fibers, but as the latter lose their regularity of arrangement, the chromatin is dispersed throughout the network (Figs. 26–27). It next breaks into small fragments which migrate to the center of the nucleus, one mass, however, (probably the heterochromosome), remaining distinct from the rest (Figs. 29–32). As the nucleus approaches maturity, it elongates and the chromatin becomes finely granular, although fragments in chain form are still distinguishable (Fig. 33).

Discussion of Results.

A number of workers (see Blackman, 1903) have described "chromatin nucleoli." They occur in a wide variety of plant and animal groups and usually during a period of growth. In oögenesis and spermatogenesis they may be present in the oö- and spermatogonia as well as in the later stages. Among the groups in which they have been discovered are echinoderms, molluscs, arthropods, amphibians and mammalians, also in protozoa and plants. It may prove instructive to compare some of the more striking of these cases with that of *Euchroma*.

Sometimes the karyosphere is formed immediately after synapsis. Illustrations of this are furnished by the oögenesis of the dragonfly, *Plathemis* (McGill, 1906), and the pollen development of *Sarracenia* (Nichols, 1908). In this plant the karyosome is formed from the synaptic threads through an absorption of the chromatin substance by a nucleolus (Plate III., Fig. 1). The achromatic substance (linin) remains as a dense mass after the chromatin has passed by drops into the closely contiguous nucleolus. There is, therefore, a rather conspicuous separation here of chromatin and linin.[1]

In *Plathemis* the synaptic threads gather closely around the nucleolus and form what Miss McGill calls a double nucleolus (oxyphil+basophil). There is plainly an interchange of material between the two parts of the nucleolus and, as the network again expands, masses of basophil substance are elaborated within the nucleolus and pass out on to the network.

In *Euchroma* the transfer of chromatic material to the karyosome is less direct, inasmuch as the synaptic threads are first extended into the network of the resting spermatocyte (Fig. 10; Plate III, Fig. 4, *a*). The latter possesses no large nucleolus such as is present in the plant nucleus. Nevertheless a center of activity arises, towards which the chromatin passes and simultaneously also the linin, at first in the form of streamers radiating from the karyosphere (Figs. 11–12). Later, as the chromosomes become distinct within the karyosphere, the linin is incorporated with them (Figs. 16–18).

Blackman (1903) interprets the karyosphere of the spermatocytic prophase of myriapods as a mass of fine, granular filaments closely gathered about the accessory chromosome. As the nucleus approaches mitosis, the threads emerge from the karosphere, shorten and thicken to become the mitotic chromosomes (Plate III, Fig. 3, *a* and *b*). There is here no separation of chromatic from achromatic substance, only a strong attraction for both to a definite region of the nucleus. As compared with *Euchroma* this tendency manifests itself much earlier, for the karyosphere has already begun to resolve itself into definite chromosomes in

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[1] A closely similar behavior of chromatin has been described for *Spirogyra* by Berghs (1906).

Scolopendra at a period when in *Euchroma* it is condensing (Plate III., Figs. 3 and 4). In *Scolopendra* there is likewise a karyosphere present in the spermatogonia, whereas it is lacking in *Euchroma*.

A somewhat different condition is described by Eisen (1900) for *Batrachoseps*. A karyosphere (chromoplast) is present in this object. At first it lies free in a vacuole, but later appears to attract "leaders" which might be compared to the radiating rays of *Euchroma* (Figs. 11–12).

Through these leaders small particles of chromatin are projected into the karyosphere and again emerge from it. The convergence of the leaders towards the karyosphere apparently corresponds to a synapsis stage, and as they finally break apart each chromosomal thread receives a portion of the karyosphere which is gradually distributed through the length of the thread[1] (Plate III., Figs. 2, *b* and *c*).

The condition in echinoderm eggs treated with Mg salts (Wilson, 1901) and in normal mouse eggs (Sobotta, 1895) is in some degree similar to that of *Euchroma*, for although there is apparently no separation of linin and chromatin, the karyosphere breaks up directly into the mitotic chromosomes (Plate III., Figs. 5, *a* and *b*).

What bearing have these facts on the question of the individuality of the chromosomes? In the paper on *Sarracenia* I suggested that the phenomena there "might be explained on the assumption that the morphological basis of the chromosomes remains in the linin while that portion of their substance which causes them to color deeply is absorbed by the nucleolus. If a similar interpretation be applied to the case of *Batrachoseps*, it will be seen that the linin retains its individuality more clearly than the chromatin, which may be transferred to the karyosphere. In the myriapods there is apparently no separation of chromatin and linin, but a tight coil of threads, consisting of both, forms the karyosphere. Possibly this might be regarded as a continuation and exaggeration of the synaptic condition, and, if this were true, a series of consecutive stages might be conceived between the typical synapsis and post-synapsis of most animals and plants and the extreme conditions presented by *Sarracenia*.

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[1] Janssens (1905) in his later work on *Batrachoseps*, remarks: "il nous semblait très probable que les chromoplastes resultaient de la soudure très intime des chromosomes aux telophases à la faveur de rapprochement." "Il semble qu'une substance intensement siderophile, une sorte de nucleine, soit venue empâter tout le pole de la figure centre à ce moment. Il se peut qu'il s'agisse là d'un exsudat des chromosomes eux-mêmes."

In the echinoderms, in *Mus* and in *Euchroma* a similar tendency to condense reappears and in the germ cells later than synapsis, owing, no doubt, to a chemical condition of the nucleus varying from the usual type. Here there is in one case (*Euchroma*) a more rapid condensation of chromatin than of linin and a consequent partial separation, while in the other (*Mus*, echinoderms) no such separation is apparent, chromatin and linin condensing simultaneously (Plate III., Figs. 4 and 5).

There is, however, little reason to believe that the difference in the method of formation of the spermatocytic chromosomes of *Euchroma* and most others insects is fundamental. The gradual change in coloration as resting chromatin becomes active goes to show that there is a chemical change in progress from less acid to more acid condition, accompanied by a condensation of substance. According to the differing constitution of different nuclei, this chemical activity might be confined to one center or distributed through the nucleus around several centers. If the latter were the case, the network of resting spermatocytes would break at various points and, condensing, form the chromosomes, but if there were but one center, the condensation would occur within a more circumscribed area. In *Euchroma*, while the latter method is more common, it may happen that the centers of condensation are multiplied (Fig. 15).

An interesting question concerning the relation of the chemistry of the nucleus to the individuality of the chromosomes presents itself at this point. If it be true that the aggregation of chromatin is accompanied by a decomposition of nucleo-proteids and a reduction of chromatin to nucleic acid or a simple compound of that acid and also true that the chromatin may be separated from the linin and gathered into a karyosphere, may it not be possible that the linin network is not homogeneous as regards its chemical character, but that in different areas are developed different proteid substances which, when combined with the nucleic acid of the karyosphere, become active and colorable by chromatin stains. It may be that usually in the development of the germ cells the nucleic acid becomes chemically dissociated, but not visibly separated from these proteid substances, and consequently no karyosphere is present, simply chromosomes consisting of a condensed linin framework surrounded by nucleic acid.

PHILADELPHIA NORMAL SCHOOL,
June 4, 1910.

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EXPLANATION OF PLATE I.

The drawings were made with the camera lucida, Zeiss microscope, oc. 12, obj. oil immersion 1/12. They have been enlarged to twice the diameter and reduced one half.

FIGS. 1–4. Spermatogonia. Prophases.

FIGS. 5–7. Last generation of spermatogonia. Prophases.

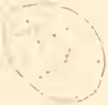
FIGS. 8–9. Synapsis.

FIG. 10. Resting spermatocyte.

FIGS. 11–18. Spermatocytes. Prophases of the first maturation division.

FIGS. 19–20. Equatorial plates of the first maturation division. 13 chromosomes.

FIGS. 21. The heterochromosome x.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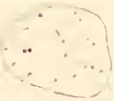
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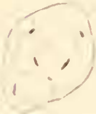
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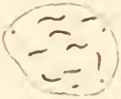
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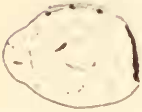
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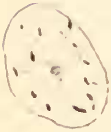
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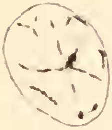
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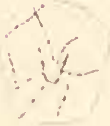
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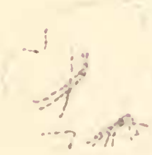
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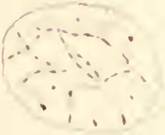
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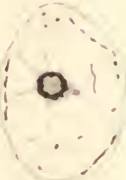
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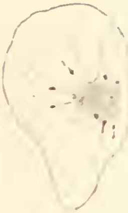
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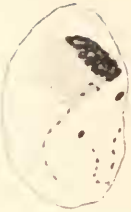
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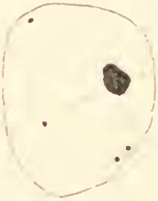
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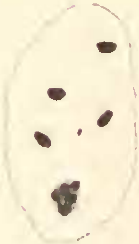
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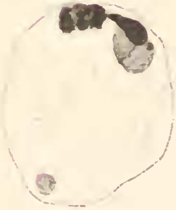
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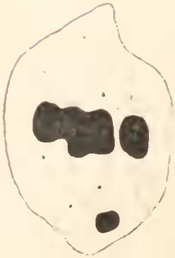
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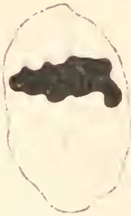
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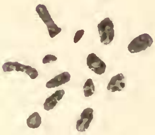
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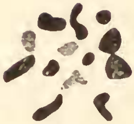
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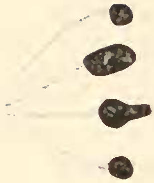

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M. LOUISE NICHOLS.

Explanation of Plate II.

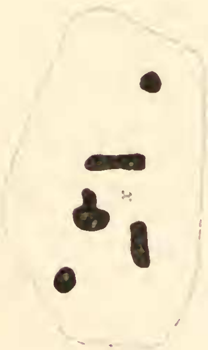
Fig. 22. The heterochromosome x.

Fig. 23. Side view of the first maturation division. Metaphase.

Fig. 24. Anaphase. Late division of the microchromosomes.

Fig. 25. Telophase. Traces of the late division of the microchromosomes.

Figs. 26–33. Spermatids.



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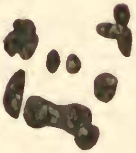

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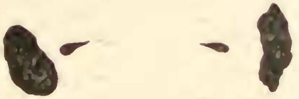

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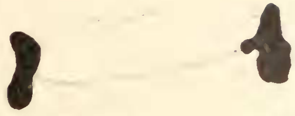

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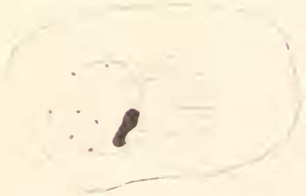

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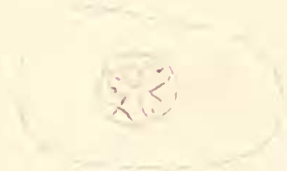

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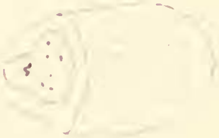

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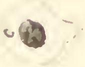

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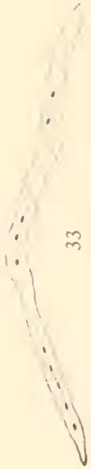

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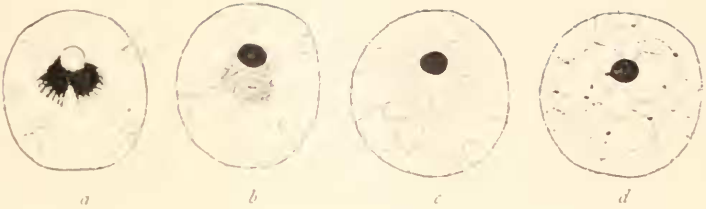
M. LOUISE NICHOLS.

Explanation of Plate III.

Figs. 1–5. Diagrams showing the relation of the karyosphere to chromatin and linin.

Sarracenia.

1



Batrachoseps.

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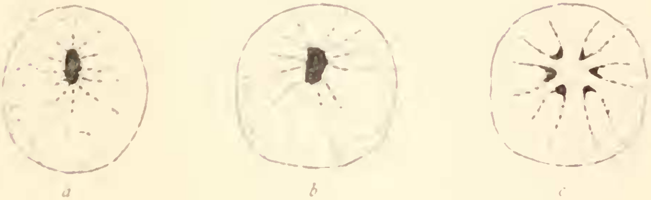

Scolopendra.

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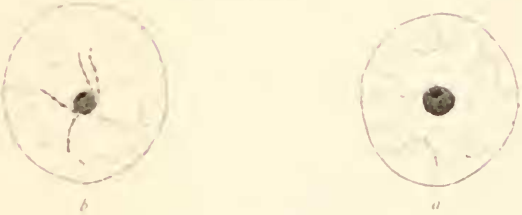

Euchroma.

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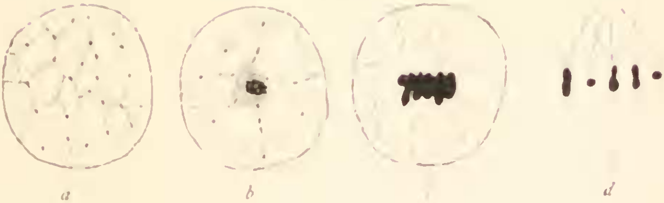
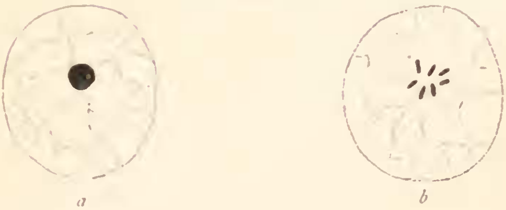


Mus. Echinoderm.

5



M. LOUISE NICHOLS.

ON THE NATURE OF THE CANALICULAR APPARATUS OF ANIMAL CELLS.[1]

R. R. BENSLEY.

During the twelve years that have elapsed since the discovery by Golgi of the internal reticular apparatus of the nerve cell, much attention has been devoted by investigators to the consideration of similar reticular structures in cells. Our knowledge has progressed along several different lines, which have been determined more or less by the techniques employed by different investigators, and although all are not agreed as to the identity of the structures so revealed, their consideration together seems to be justified by the great similarity in configuration and location which these elements possess.

It is not necessary in this paper to review in extenso the literature on this topic, for that is well covered by the summary given by Holmgren ('02) in Merkel und Bonnet, Ergebnisse, etc., in which also the different theories of the nature of these structures are well brought out. The more recent contributions are considered in the article of von Bergen ('04) and that of Legendre ('08-'09). It will suffice here to summarize the progress that has been made along the different lines of investigation, and to consider the interpretations of these structures which have been advanced by different workers.

Golgi ('98) first described the internal reticular apparatus in the cells of Purkinje of the cerebellar cortex, where he demonstrated it by means of a modification of his well-known chrome silver impregnation method. He describes it as a closed net of fine fibers occupying the intermediate zone of the cell protoplasm and separated by a distinct interval from the nucleus on the one hand and from the surface of the cell on the other, that is to say, there was a zone of protoplasm on the periphery of the cell which was wholly free from the fibers constituting the network. Toward the nucleus the net sent out fine fibers so that the perinuclear space was not wholly devoid of fibers.

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[1] From the Hull Laboratory of Anatomy, University of Chicago.

In his later studies Golgi confirmed this result for other types of nerve cells including spinal ganglion cells, spinal cord cells and cells of the cerebral cortex. In some of these cases he found the network provided with freely ending branches which terminated in a small swelling. In some cases, too, the fibers of which the network was composed had varicose swellings on them, and nodal enlargements, and, in some cells, he even found two concentric nets which differed inter se by the amplitude of the meshes and the size of the fibers. Golgi in all of his papers expressed himself with great reserve as to the nature of the network, but was confident that they had nothing to do with the neurofibrils and that they were not canals which had been filled with the silver precipitate. He was moreover certain that they were entirely intracellular and that they had no communication with extracellular structures.

In the meantime Golgi himself and his students had been extending the field of investigation to other than nervous tissues, and it had developed from these investigations that the reticular apparatus was not confined by any means to nerve cells but was present in a large variety of cells from different sources. For example, Negri ('00) demonstrated an apparatus of this sort in the cells of the pancreas and of the parotid gland of the cat, and in the thyroid epithelium of the dog. In these cells the situation of the network was quite characteristic and recalled the observations of Golgi that in young nerve cells with excentric nucleus the reticular apparatus was also excentric and located for the most part at one pole of the nucleus. In the epithelial cells, namely, it was found that the reticular apparatus was located near the nucleus, but between the latter and the free border on the lumen or surface, that is, it was distal to the blood vessels. Later, similar nets were found in the cells of the epididymus by Negri, in cartilage cells by Pensa ('01), and in striated muscle fibers by Veratti ('02).

The observations of Golgi were confirmed by a number of observers, using this method or one of the silver reduction methods of Cajal. Retzius, for example, obtained good impregnations of the apparatus in the nerve cells of the cat and rabbit, which corresponded in their salient characters with those of Golgi but were not nearly so complete judging by the figures published. In view of this it is surprising to note that Retzius ('00) found, in some of the cells, the fibers of the network communicating by a branch with the surface of the cell, which Golgi had never observed in his more perfect preparations.

In a recent publication Golgi has adopted a new method for the demonstration of the reticulum which is based on the silver reduction method of Cajal. In view of this fact it is proper to mention here that Cajal ('07) has also studied the reticular apparatus in the nerve cell, which he accepts, contrary to Golgi, as a tubular apparatus to which he applies the name "Conduits de Golgi-Holmgren," thus accepting the interpretation of Holmgren that they are the same as the so-called juice canals described by him. He regards the appearances seen in his preparations as due to the presence of canals filled with a coagulable substance which has an affinity for colloidal silver. He also notes differences in the behavior of the apparatus in different animals, from which he concludes that the contents of the canals in different cells may have different chemical properties.

By the same methods as those employed by Cajal, Sanchez demonstrated an exceedingly interesting system in the striated muscle fibers of mammals and insects. In the former this system did not communicate with structures outside of the cell but sent free ending branches which terminated just under the sarcolemma. In the insects, however, he made the surprising observation that the intercellular network was in continuity with the tracheal system.

The second line of progress in the study of the reticular apparatus began with the discovery by Kopsch that it could be stained by prolonged immersion of the tissues in a two per cent. solution of osmic acid. In a short paper (Kopsch, '02) he described his method and contributed the results of his application of it. The results obtained corresponded very closely to those of Golgi but were obtained with greater certainty. Like Golgi he was unable to find any communications between the apparatus and the surface of the cell, although in addition to the osmic acid method he employed the resorcin-fuchsin method of Holmgren, to which reference will be made later.

The method of Kopsch has been exploited in particular by Misch ('03), and von Bergen ('04). The former found that the apparatus was not present in all cells and that in some cells it presented itself in the form of fragments, or of rows of granules. He found, moreover, in agreement with Golgi and Kopsch that the network never communicated with the surface of the cell, nor did it penetrate the nucleus. Von Bergen's studies extended to a very large category of cells ranging from wander cells to nerve cells. To show how general these structures are in animal cells a list of the elements in which von Bergen obtained positive results would have considerable interest. In addition to nerve cells he found a reticular apparatus in the following elements: prostate epithelium, pancreas cells, demilunes and mucous cells of the submaxillary gland of the cat, glandular epithelium from the trachea, chief cells of the fundus glands of the stomach, ciliated epithelium of the trachea, epithelium of the sweat glands, wander cells and many leucocytes, fixed connective tissue cells, cartilage cells, endothelium, smooth muscle, interstitial cells of the testis. The wide range of these observations taken in connection with the observations of Golgi and Cajal and their pupils indicate that the reticular apparatus is by no means a structure confined to a single cell category but is a cell organ of almost if not quite universal occurrence in the.protoplasm of animal cells.

Before passing to a review of the investigations that have been made from the standpoint of the canalicular apparatus of Holmgren and others it may be of interest to refer briefly to the studies of Golgi on the development of the reticular apparatus. In the nerve cells of the fœtal calves of two or three months, he found the apparatus greatly reduced, often consisting of but a single fiber, with short branches running in various directions. In these cells the apparatus has a distinctly excentric position at one pole of the nucleus. In the new-born animal the net often extended around the nucleus, but left the perinuclear zone as well as the peripheral protoplasmic zone entirely free of such fibers. In old animals the apparatus was sometimes broken up into peculiar island-like fragments which however were connected with one another by single fibers. These observations suggest strongly that the apparatus constitutes a unit in its origin and developmental history.

The history of the intracellular canalicular apparatus, considered apart from the positive impregnations of Golgi and his followers, begins with the discovery by Holmgren ('99) of endocellular nets of juice-canals in nerve cells which he said was exhibited particularly well in preparations made from rabbit tissues. Almost at the same time Nelis ('99) described, in nerve cells fixed in sublimate or osmic acid and stained in iron-hæmatoxylin, etc., peculiar coil-like bands to which he gave the name "état spirémateux," the nature of which, however, remained to him fully obscure.

In a second publication (Holmgren, '99, 2) Holmgren described in greater detail the canalicular apparatus in the spinal ganglion cells of the rabbit, fixed in picric acid-sublimate and stained with toluidene blue and erythrosin. He found in these cells moderately fine canals of fairly uniform caliber which, anastomosing freely, formed a fairly dense network. The latter extended in general around the nucleus but often was found at one pole of the nucleus, more rarely at both poles. Here and there he found these canals communicating with pericellular canals, and at these points he was able to make out a distinct wall staining with erythrosin. He expressed the opinion that the canals were of lymphatic nature without however stating whether they were of extracellular or intracellular origin.

In 1899 Studnicka ('99) also described the canals in the protoplasm of the large ganglion cells of the trigeminus of *Petromyzon* and also in the spinal ganglion cells, in the nerve cells of the medulla oblongata and the cells of Reissner of the same animal. He explained the origin of the canals as due to the union of a row of vacuoles, and said that while many of the canals had smooth contours, yet in others might easily be seen the constituent vacuoles from which they had arisen. In a foot-note he remarked that he had not found in his objects the connection with extracellular structures described by Holmgren, although he admitted that the canals opened on the surface into the pericellular space.

In a series of papers dating from 1899 Holmgren has described the results of his investigations on this topic, covering a wide range of material including not only nerve cells, but cells from various epithelia and from other sources. The existence of the canals has also been confirmed by a large number of observers including Kolster ('00), Fragnito ('00), Lugaro ('00), Donaggio (—), Pugnat ('01), Sjövall ('01), Smirnow ('01), von Bergen ('04) and others.

For comparison with the results of the Golgi and Kopsch techniques an enumeration of the different types of cells in which a canalicular apparatus has been found may be of interest. Holmgren demonstrated the canals in the following cells: gland cells of the pancreas and parotid, intestinal and gastric epithelium, epithelium of the epididymus, biliary duct epithelium, uterine epithelium, thyroid epithelium, liver cells, epithelium of the suprarenal gland. Retzius ('01) described similar canals in the giant cells of the bone-marrow, which, like Holmgren, he considered to be in direct connection with pericellular spaces.

It is to be noted that many of the objects studied by Holmgren coincide with those studied by Golgi and his pupils, and with those investigated by von Bergen, and that where this is the case, the canalicular structures described by Holmgren correspond closely in their location and in their configuration with those demonstrated by the other methods. Whatever conclusion we may reach with regard to the relation between the canalicular apparatus of Holmgren and the reticular apparatus in nerve cells, few who have studied the actual preparations made according to these different techniques in respect to epithelial cells and cartilage cells will deny their substantial identity. It is true that there are differences in the appearances obtained, but, in the opinion of many, these are sufficiently accounted for by the differences in the thickness of the sections studied in the different methods, and so, in the completeness of the apparatus which is brought to expression in a single preparation.

In his later papers dealing with these structures Holmgren has abandoned his original opinion that the canals are lymphatic in nature and constructed an entirely new hypothesis as to their nature. This hypothesis is based on the confirmation by him of the interesting observations of Nansen ('86) and Rhode ('91, '93, '95), that the nerve cells of certain Crustacea (Nansen, '86), and those of certain Gastropoda and Hirudinea (Rhode, *loc.*

cit.) were penetrated by a network derived from surrounding capsular cells. Holmgren found the nerve cells of *Helix pomatia* particularly suitable for the demonstration of these intracellular nets of capsular origin. He found here that the nerve cells were provided with a richer or poorer network of juice-canals, which were formed in the interior of a network of processes derived from other cells. He even found nucleated strands within the bodies of the nerve cell. In later publications (Holmgren, '01, '02, etc.)[1] he has developed this hypothesis on the basis of results obtained by the employment of a new method. He fixed his material in trichloracetic acid, or trichlorlactic acid, and stained it with a freshly prepared solution of Weigert's resorcin-fuchsin. By this method the protoplasm of the nerve cells stained faintly but that of the intracapsular cells stained dark violet, as did also the processes of the latter. By this means he was able to see processes of the darkly stained intracapsular cells which penetrated the nerve cells, branched within them, and anastomosed with one another, in order to produce an intracellular network. He applied this observation also to the nerve cells of vertebrates, and came to the conclusion that the latter were penetrated by processes of other cells which branched and anastomosed freely, to form in the interior of the nerve cells a "spongioplasma" which, however, in no wise belonged to the nerve cell, but was of extraneous origin. In the interior of these nets juice canals could arise, which communicated directly with similar spaces in the interior of the matrix-cells of this net. To this net of extraneous origin Holmgren gave the name "trophospongium." He regarded therefore the trophospongia not as fixed structures, but as undergoing a constant change, which depended upon the physico-chemical processes in the cell, and thought that, while at one moment the network of cell processes might sacrifice itself by liquefaction to the needs of the nerve cell, it might later be regenerated, by new growth of the process from without. He thus abandoned completely his former view that the canals represented circulatory or lymphatic structures, or a drainage system, in favor of the view that they represented the transitory phases of the reciprocal nutritive inter-relations of the capsular and nerve cells.

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[1] For complete references see Holmgren, E., "Neue Beiträge zur Morphologie der Zelle," in "Merkel-Bonnet Ergebnisse," Vol. 11, p. 275.

The object of the foregoing brief and incomplete résumé of the literature, has been to show that from three different lines of investigation we have evidence of the wide occurrence in animal cells, ranging in diversity from leucocytes to nerve cells and muscle cells, of a reticular apparatus, which exhibits itself in the form of a network of canals with colorless contents, or of a stained network according to the technique employed. The uniformity with which this apparatus has been discovered in those types of cells in which it has been sought justifies the expectation that similar methods will reveal similar structures in cells which have thus far not been investigated with this point in view. We are thus dealing with a cell organ of almost if not quite universal distribution in animal cells.

The question now arises—What is the significance of this structure?

The trophospongium theory of Holmgren, as far as I am aware, has found no support. Even if it were admitted for the nerve cells there are many categories of cells in which a reticular apparatus, or a canalicular apparatus is to be found, to which the theory is wholly inapplicable. For example, it is difficult to conceive how the reticular canalicular apparatus of the cartilage cells and of leucocytes, could be derived from the liquefaction of penetrating processes of other cells. Holmgren, it is true, has made an attempt to adapt his hypothesis to the canalicular apparatus of epithelial cells, and has described in the pancreas of the salamander the continuity of the intracellular network with intracellular strands which go to the periglandular connective tissue cells or to the centro-acinous cells. However, all of Holmgren's figures of preparations made by the trichloracetic acid, resorcin-fuchsin method are explainable on the basis of the canals having a precipitable content which when precipitated by the fixative, has an elective affinity for the dye. It is not by any means certain that the figures which Holmgren has given us of intracellular nets stained by fuchsin, in continuity with processes of capsular cells, do not really represent two different structures brought into apparent relation with one another by a common affinity for the dye. It is certain, moreover, that the networks apparently composed of solid fibers demonstrated in the pancreas epithelium by the resorcin-fuchsin method, are not solid, for, in preparations made by this method, every cell in the section will show such a deeply stained network while sections of the same pancreas fixed in Kopsch's formol-bichromate solution and stained with iron-haematoxylin, will show in every cell a system of canals with unstained contents.

Accordingly, we must either reject Holmgren's hypothesis or assume that there are two sorts of these nets, those of cartilage cells and epithelial cells and leucocytes being different from those of nerve cells.

The statement of Legendre ('08-'09) that these structures are either wholly absent or are the result of pathological changes is not to be seriously considered, in view of the fact that this author in attempting to explain the positive observations in this regard of so many experienced investigators, is compelled to resort to the wholly unwarranted assumption that their results have been due to the selection of unhealthy animals, or to the fixation of tissues after several days of inanition, or several hours after death.

Many observers, including Retzius ('00), are inclined to believe that they represent an intracellular system of nutritive or drainage canals having direct relations with the lymphatic system. The extracellular communications are, however, denied by Golgi and his pupils, who have never seen them in their preparations made by the chrome-silver impregnation methods, nor by the silver reduction method. They are equally denied by von Bergen, who, however, admits the existence of canals in no wise connected with these, which he regards as artefacts, which do open on the surface of the cell.

Von Bergen ('04) who studied these structures by all three methods but, in particular, by the osmic acid method of Kopsch, agrees with Golgi that the structures are for the most part networks of fibers composed of a substance which reduces osmic acid, but explains the discontinuous elements found by him in many cells studied, by the assumption that they represent different stages in the formation or destruction of the apparatus which thus would have a variable structure from moment to moment in the cell. He claims that the reticular apparatus arises by the appearance in the cell protoplasm of granules or droplets which arrange themselves in net-like or tortuous rows which fuse to form more continuous fibers, and further, that the network so formed can undergo vital changes, by virtue of which it loses its stainability and becomes dissolved, the canals so formed finally disappearing by absorption of their contents.

In view of the almost universal occurrence of these structures in all of the tissue cells of mammals, and in many of those of lower vertebrates and invertebrates, it seemed probable that they would not be wholly absent from the cells of the other great division of living organisms, namely from the cells of plants. Accordingly, I have studied with this end in view the structure of certain plant cells, using for this purpose in addition to the conventional methods of plant histology, those methods which in my experience were best for demonstration of the canalicular system in animal cells. It seemed probable, in view of the conditions found in the animal cell, that, if a homologue of the canal network of the animal cell were to be found in plant cells, it would be studied with greatest ease in those plant cells in which the vacuolar system had not yet reached its full development, namely in meristem tissues, sporogenous tissues and their products, cambium and embryonic tissues. The three last, however, did not lend themselves readily to this investigation because of the difficulty introduced by the slow penetration of the fixing agents, so that I have been obliged for the present to content myself with the results obtained in the root-tips of *Allium*, *Lilium* and *Iris*, and in the tapetum of the lily. Whether the consistent results obtained from the study of these cells are generally applicable or not to plant cells, future investigation will show. In the meantime, because of the fact that the results are at variance with the accepted views of the structure of the cells in question, because they furnish a new interpretation of the history of the vacuole of these cells and in particular because they seem to throw an interesting light on the question of the nature of the canalicular apparatus of animal cells, it seems wise to put these preliminary observations on record.

The descriptions which follow have been drawn largely from the study of preparations of the root-tip of the onion, but the observations made on the roots of the other genera mentioned are in full accord with them.

In his study of the vacuolar system of the cells of plants, Went ('88) describes the young cells of the onion root-tip as follows: "In the youngest cells of roots two or three millimeters in diameter I saw a great number of very small vacuoles; the largest had a diameter of four mikra, the smallest of one mikron." For these small vacuoles he claims reproduction by division, in the sense of the tonoplast theory of DeVries ('85). He also derives the vacuole of the older cell from these multiple vacuoles, by a process of coalescence.

In preparations made after fixation in Flemming's strong fluid, Hermann's fluid, Zenker's fluid, Carnoy's fluid, etc., and stained in iron hæmatoxylin, or in the three-color process of Flemming, results exactly corresponding to these were obtained, that is to say, the young cells contained a multitude of small vacuoles which by their coalescence seemed to form the large central vacuole of the older cell.

On the other hand, preparations made by methods which I had found to be most effective for the demonstration of the canalicular apparatus in animal cells, gave results which were wholly different. In these there was no trace in the youngest cells of the root tip of the multiple vacuoles described by Went and others, but instead, each cell possessed an intricate network of canals the component elements of which in the youngest cells were often of extreme fineness. These canals were best seen in the dermatogen cells on the surface of the root but were recognizable as such, though less well preserved, in the cells of the plerome. Fig. 1 shows a cell in which this system is composed of extremely fine canals. In this figure it will be seen that the canalicular system tends in these cells as in the animal cells to

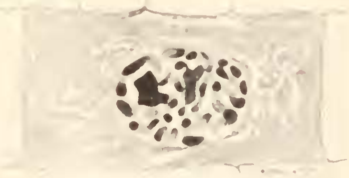

Fig. 1. Cell of outer layer of the root tip of onion, showing fine canals in the cytoplasm. × 800.

leave a peripheral zone of cytoplasm wholly free from the canals which constitute it. In Fig. 2 are shown four of the large wedge-shaped cells from the region of most active division of the dermatogen, in which the type of the canalicular system is well brought out. Here it will be seen that in these plant cells, as in the animal cells, the network tends to be concentrated on one side of the nucleus, and, as in the epithelial cells, this point of concentration is not one of the division poles of the nucleus but corresponds to one side of the equator of the future spindle. The system constitutes a closed system of canals, lying in very close relation to the nucleus, never, however, invading, in dividing cells, the spindle territory. From this network run out branches which end freely often near the cell wall in a small expansion. Many of the canals in some preparations, and this is particularly true of the smallest canals, such as those shown in Fig. 1, show moniliform enlargements, as if they were on the point of breaking up into a row of vacuoles, or possibly, as if they had just been formed by the coalescence of a row of vacuoles. Again frequently, the canals show a spiral or tortuous course, as if they were fixed while in a condition of internal tension, which resembles very closely the spiral or tortuous condition found in many nerve cells (état spirémateux of Nelis).

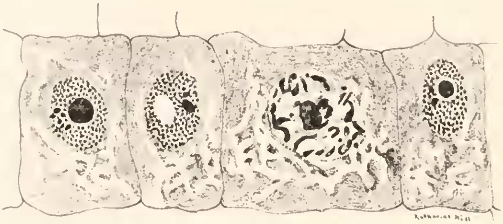

FIG. 2. Four cells of outer layer of root tip of onion, showing more advanced condition of the cytoplasmic canals. × 800.

Tracing this system in the older and older cells of the root tip it is found that as the cell retreats from the growing point, the canals become progressively larger and larger. In the intermediate stages of this process the condition depicted in Fig. 3 is obtained. Here there is still a continuous system of canals but they are fewer in number and broader than in the younger cells. Ultimately by a continuation of this process we have the familiar picture of the plant cell with a large central vacuole across which run strands of protoplasm which are the last attenuated remains of the protoplasmic partitions between the canals.

A similar mechanism is revealed by the same technique in the tapetal cells of *Lilium candidum.* In preparations made after fixation in Flemming's fluid, the protoplasmic tip of the cell presents a foam-structure owing to the presence in it of a large number of fine vacuoles. In preparations, however, which have been made by the technique referred to above, it is seen that instead of a multitude of minute vacuoles or alveolæ, there is a system of exceedingly fine canals forming a network which opens at intervals into the large vacuole which occupies the base of the cell.

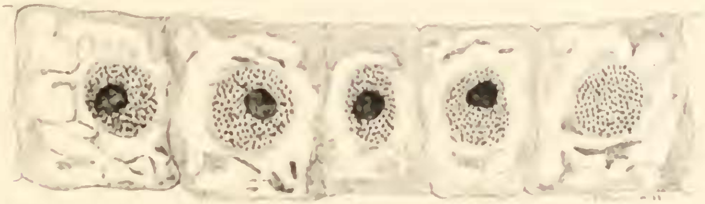

FIG. 3. Five cells of second row of root tip of onion, showing the expanded canals, beginning to form the large central vacuole.

Apart from the elements which constitute the tubes or vacuoles according to the method of preparation, the cytoplasm of these cells shows no indication whatever of an alveolar structure under the microscope. It is composed of an optically homogeneous ground substance in which are imbedded the mitochondria and other granular elements, for example plastids, which may be present.

The two different techniques, therefore, give us two entirely different conceptions of the history of the vacuole. According to the first the vacuole arises from the coalescence of preëxisting innumerable small vacuoles. According to the new methods, the vacuole in these cells constitutes a unit element from the very beginning, being represented in the younger cells by a single system of anastomosing canals.

The decision as to which of these views expresses the condition in the living cell must of necessity rest on the examination of living cells. Before taking up this question, however, we may discuss the significance of these observations in the interpretation of the canalicular apparatus of the animal cell for this interpretation does not of necessity imply the assumption that the canalicular structure so demonstrated in the plant cell has a real preëxistence in that form in the living cell. We may, on the contrary, treat the technique as an experimental method and discuss the results comparatively on this basis.

For the demonstration of the vacuolar system of plant cells as a network of canals I have found the following fixing fluids best adapted:

1. FORMALINE, BICHROMATE, SUBLIMATE.

Neutral formaline (freshly distilled)	10	c.c.
Water	90	c.c.
Potassium bichromate	2.5	gr.
Mercuric chloride	5	gr.

2. KOPSCH'S FLUID.

Potassium bichromate 2.5 per cent. in water	75	c.c.
Neutral formaline	25	c.c.

With these fluids, as indicated above, the cells of the root tip show a network of canals, whereas the same tissues fixed in Flemming's solution show, instead of canals, multiple small vacuoles. The same statement holds good for animal cells similarly treated. For example, the epithelial cells of the intestinal glands fixed in the formaline-bichromate-sublimate mixture, or in Kopsch's fluid, show a beautiful canalicular system, while the same cells fixed in Flemming's fluid show at the site of the canals merely a large number of exceedingly fine vacuoles. Thus whether we accept the multiply vacuolated condition, or the canalicular condition, as the preëxisting one in the living cell, the analogy between these structures in the animal and vegetable cell holds.

On the basis of the similarity in constitution of the canalicular apparatus of the plant cell to that of the animal cell, and of the similarity in behavior of this system when treated by the same methods and an account of the part these canals in the plant cell take in the history of the vacuole of the latter, I think we are justified in stating for the present, to be sure, only as a working hypothesis, that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system of the plant cell.

We may now return to the consideration of the question whether the canalicular system represents the true structure of the vacuolar mechanism of young plant cells or not. This question can, of course, be answered only by observations of the living cells themselves, and the investigation of these is beset by extraordinary difficulties in the case of the plant cell due in particular to the impossibility of finding a solution in which to examine the cells, which is not itself injurious. In the effort to find a suitable fluid for this purpose I tried solutions of potassium nitrate, of sodium chloride, and of cane sugar of different concentrations, but found in all that the surface layers of cells showed rapid changes of the structure of the protoplasm which made it difficult to study the presumably uninjured deeper layers of the sections. I was finally obliged to resort to the expedient of using as a mounting medium the freshly expressed juice of similar tissues, although even in this the cells of the surface layers of free-hand sections underwent more slowly the same change. In these surface layers so mounted the cells showed the multiple vacuolar condition described by Went. In the deeper layers, on the contrary, in sections of the onion root tip, one could see, with difficulty to be sure, but still unmistakably the canal system represented in Figs. 1, 2 and 3. As these cells are watched, however, the canals are seen to break up slowly into rounded vacuoles thus bringing about the condition generally recognized in these cells. I regard, therefore, the canalicular system as the true condition intra vitam of the vacuolar apparatus in these cells of the root tip, and believe that the multiply vacuolated condition is of secondary origin due in most cases to injury of the cell.

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A STUDY OF CHROMOSOMES OF TOXOPNEUSTES VARIEGATUS WHICH SHOW INDIVIDUAL PECULIARITIES OF FORM.

BARBARA HEFFNER.

INTRODUCTION.

The observations described in this paper were made during the winter and spring, 1909–10, in the Biological Laboratory of Bryn Mawr College.[1] Last November after my arrival in Bryn Mawr Professor Tennent suggested to me a study of the chromosomes in eggs of certain echinoderms with special refence to characteristic peculiarities of form, a question especially significant since the appearance of Baltzer's ('09*a*) paper on the chromosomes of *Strongylocentrotus lividus* and *Echinus microtuberculatus* which has thrown new light on the individuality of chromosomes in echinoids. Previous to this author's first publication on the subject ('08) there existed only a suggestion by Boveri ('01 and '07) that in some echinoids there occur chromosomes of a characteristic shape. Baltzer pointed out that in *Echinus microtuberculatus* there are two very long rod-shaped chromosomes, two long hook-shaped ones, and two or three horseshoe-shaped chromosomes, while in *Strongylocentrotus lividus* there are two long rod-shaped chromosomes, two long hook-shaped ones and in a part of the eggs one smaller hook-shaped chromosome. As this latter one occurs only in a part of the eggs—in about one half of them—Baltzer suggests ('09*a*, p. 596) that it is probably an idiochromosome whose smaller mate is one of the shorter rod-shaped chromosomes. The same suggestion is made in regard to one of the horseshoe-shaped chromosomes in *Echinus*, in cases where there are three of that type. While idiochromosomes and other heterochromosomes have for some time been known in insects, arachnids and myriapods, they have only quite recently been discovered by Gulick in the nematode *Heterakis* (Boveri, '10), by Baltzer in the echinoids, and by Guyer ('09*a* and *b*) in vertebrates.

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[1] I take advantage of this opportunity to express my sincere thanks for the generous scholarship awarded to me by Bryn Mawr College and for encouraging suggestions from Professor Tennent and Dr. Stevens during the course of my work in their laboratory.

Observations on metakinesis stages (Baltzer '09*a*, Plate XXXVII., Fig. 9) have shown that the hook-shape of certain chromosomes in *Strongylocentrotus* is due to the fact that the spindle fiber from each pole is attached at a point about one third of the length of the chromosome from one end, so that a shorter and a longer arm are formed. In the horseshoe-shaped chromosomes in *Echinus* the fiber is attached about half way between the two ends, so that the two arms are nearly of the same length (*ibid.*, Fig. 10). As for the origin of the hook-shaped and horseshoe-shaped heterochromosomes, observations on cross-fertilized eggs, $\frac{\textit{Strongylocentrotus}\ ♂}{\textit{Echinus}\ ♀}$, and on multipolar mitoses of *Strongylocentrotus* have shown that they come from the female pronucleus, the corresponding pair in the male being rod-shaped. Apparently the female has an unequal pair of heterochromosomes, one hook- or horseshoe-shaped, the other rod-shaped; while the male has a corresponding equal pair of rod-shaped chromosomes. It may be mentioned that this is the reverse of what is found in insects, but as in most insects the female nucleus must obtain more chromatin than the male nucleus.

Material and Methods.

My observations were made upon eggs of *Toxopneustes* and *Arbacia*, collected and preserved by Professor Tennent. As preserving fluid either picro-acetic or sublimate-acetic was used. Sections of 5 μ thickness were stained with Heidenhain's iron hæmatoxylin, except in a few cases mentioned later.

The figures are all drawn with Abbe's drawing camera, Zeiss oil immersion 2 mm. apochr. objective, oc. 12, enlarged to twice or four times the original diameter and reduced one half.

Arbacia punctulata.

The eggs of this species are quite unfavorable for detailed cytological studies. Not only are the chromosomes very small but the cytoplasm of the egg is filled with pigment granules so that a sharp differentiation of plasma and chromosomes is impossible. Following Dr. Stevens's suggestion, I tried to bleach the section with H_2O_2, a method successfully applied in some other cases, but entirely useless in *Arbacia*. I therefore gave up further study of the chromosomes of this species.

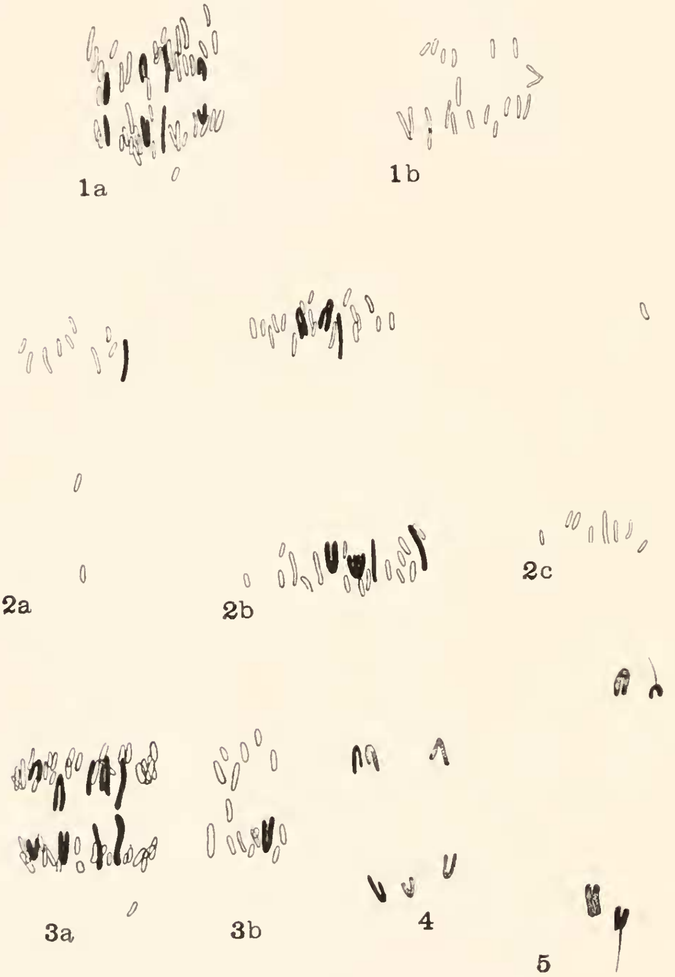
TOXOPNEUSTES VARIEGATUS.

The observations were made on two series of eggs from two different localities, one from Beaufort, N. C., the other from the Tortugas. The results obtained are the same for both. I began with the study of the chromosomes in the first segmentation spindle and found in *Toxopneustes* as Baltzer ('09*a*) had done in *Strongylocentrotus* and *Echinus* a considerable variation in the length and form of the chromosomes.

As in *Echinus* and *Strongylocentrotus* there are two extremely long chromosomes in each daughter plate. Their behavior resembles that described by Baltzer in that these long rod-shaped chromosomes are often late in splitting and moving to the poles (compare Text-fig. 3, *a*, with Baltzer's '09*a*, Pl. XXXVII., Fig. 5, *a* and *b*). These chromosomes may also be seen in my Figs. 1, 2 and 3. Sometimes these long chromosomes are contracted more and then appear thicker and shorter (Fig. 1, *a*, the left pair of long rod-shaped chromosomes).

A type of chromosome of peculiar shape, found in all of the eggs, is one which is usually V-shaped, but sometimes more horseshoe-shaped (Figs. 4 and 5). Very frequently in late anaphases the two arms are parallel or nearly so, and one may partly overlie the other, but there is hardly ever any doubt as to whether there is one chromosome with two arms present or two separate rod-shaped ones (Fig. 3, *a* and *b*). As may be observed from Fig. 4 the length of the two arms in these chromosomes may vary slightly when the arms are lying in one optical plane. I tried to determine whether this difference is confined only to certain chromosomes or to chromosomes in certain eggs, but no regularity seems to exist. The probable origin of the difference in length will be discussed later.

These V-shaped chromosomes occur in all fertilized *Toxopneustes* eggs and there are either two or three present. Among 34 one-cell stages examined with reference to this point, I found in 16 cases two, and in 18 three such chromosomes. Fig. 1, *a*, illustrates a case with two V-shaped chromosomes in each daughter plate. Their antagonistic position proves that they are division



Figs. 1–5.

products of the same chromosome, their regular number, that they are not merely incidental features. The other chromosomes are very crowded, as most of them occur in one section. Their position has been slightly changed in cases where they were over or under the V-shaped chromosomes, and this holds for all similar figures. Special care was of course taken to keep the position of the long rod-shaped and the V-shaped chromosomes in every case as accurately as possible.

Fig. 2, *b*, shows daughter plates of a late anaphase where there are three V-shaped chromosomes with more nearly parallel arms. Two of these chromosomes are very close together, one partly overlying another in each plate. Fig. 3 also shows in *a* and *b* three V-shaped chromosomes in early anaphase. One pair of these chromosomes appears smaller than the two others, but in examining other cases I found no regularity in the apparent size of the three pairs. Sometimes all three pairs vary a little, sometimes they are all of apparently the same size, sometimes only the members of one pair vary in apparent size. This difference may be due to difference in contraction or to an original difference in length of the chromosomes. As Baltzer's ('09*a*, p. 568) measurements of the hook-shaped chromosomes show, the length of chomosomes of a certain type is quite variable; it may vary from 9.75-14.0 mm. There is no resemblance however to the conditions in *Strongylocentrotus* in respect to the third pair of hook-shaped chromosomes which always is considerably smaller than the other two.

One of the three V-shaped chromosomes is probably a heterochromosome, as Baltzer assumes to account for the conditions found in *Strongylocentrotus* and *Echinus*. Studies in ovogenesis and spermatogenesis would be necessary to obtain evidence for or against the suggestion made.

As it is difficult to count the total number of chromosomes in a lateral view of the daughter plates I counted the number in polar views where fortunately the V-shaped chromosomes show very clearly.

Fig. 6, *a* and *b*, represents two succeeding sections through two anaphase daughter plates; the V-shaped chromosomes are the two double ones, finished in solid black. The number of chromosomes in each plate is 36. Fig. 7 shows a polar view with three V-shaped chromosomes; here also the number is 36.

I counted 14 anaphase plates from the pole, with a clear arrangement of chromosomes, and found the average number

36, always counting the two arms of the V-shaped chromosome as one.

Although I did not have very much material I was nevertheless able to trace the V-shaped chromosomes in the 2-, 4-, 8-, 12- and 16- to 32-cell stages.

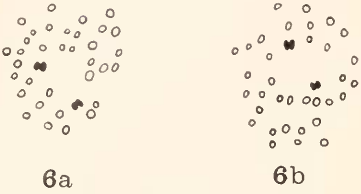


Fig. 6.

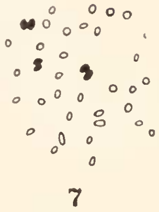


Fig. 7.

Fig. 8, *a* and *b*, shows a metaphase from a 2-cell stage, with the three V-shaped chromosomes distinguishable by their characteristic splitting figures, which are fully explained below.

Fig. 9, *A* and *B*, represents two adjoining cells of a 16- to 32-cell stage. In cell *A*, 9*b*, we see three V-shaped chromosomes and the two long ones. In *B*, 9*c*, we see again two of the V-shaped chromosomes; and in 9*d* the third one. The two long ones are distributed between *c* and *d*. The chromosomes in this stage are so small and crowded together that an accurate count of their total number is impossible.

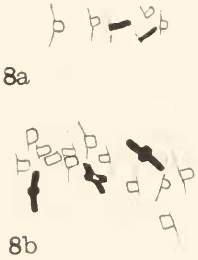


Fig. 8.

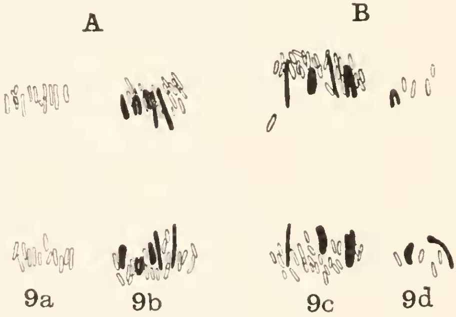
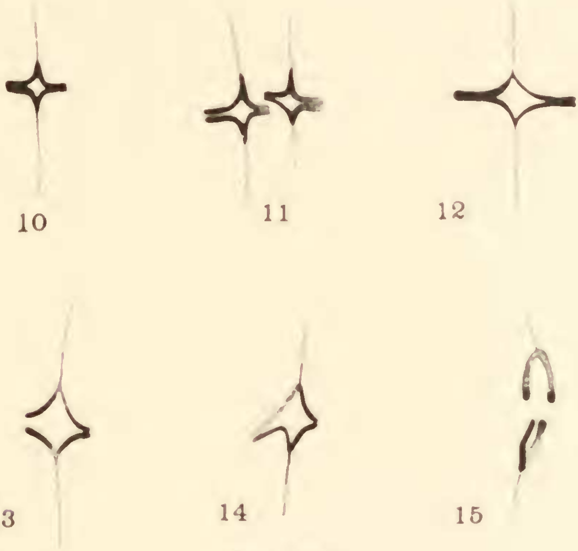


Fig. 9.

The figures 10–15, enlarged 4 diameters and in publication reduced one half, show splitting, or metakinesis, stages of the

V-shaped chromosomes. From these figures it is evident that the V-shaped chromosomes of the anaphase come from a long rod-shaped chromosome to which the spindle fibers are attached with more or less regularity in the middle of the chromosome (Fig. 12). If the separation of the daughter-chromosomes takes place earlier at one end than at the other (Fig. 13) or one arm of the chromosome is contracted more than the other, or, as Fig. 14 suggests, if some hindrance on one side prevents one end from moving toward the pole as rapidly as the other end, then the variations in the length of the arms mentioned above (Fig. 4) appear. In comparing these figures with Baltzer's '09*a*, Pl. XXXVII., Fig. 10, we cannot fail to find a close resemblance.



FIGS. 10–15.

In Baltzer's paper '09 *a*, p. 607, we notice a suggestion that in the hook-shaped chromosomes there may be a union of two chromosomes, end to end at the point where spindle fibers are attached. The reasons given for this suggestion are the extraordinary length of the hook-shaped chromosomes, and the fact that all other chromosomes are attached to the spindle fibers by one end. This suggestion is a very natural one, for such apparently homogeneous but plurivalent chromosomes are known in *Ascaris*, and compound chromosomes are found in the maturation mitoses of certain insects (McClung, '05, Payne, '09). In some of these latter cases (Payne, '09) the plurivalent chromosome has a spindle fiber attached to each unit in the early metaphase and in many cases two spindle fibers from each pole are attached to one of each of the four units of a tetrad in primary maturation mitoses (Stevens, '10).

Since the V-shaped chromosome of *Toxopneustes* seems to be exactly comparable to the hook-shaped chromosome of *Echinus* and *Strongylocentrotus*, the question arose whether, assuming that the V-shaped chromosomes of *Toxopneustes* may be bivalent, one might by careful observation be able to trace in the early metaphase two spindle fibers from each pole attached to each of them. As the spindle fibers were not especially clear in the preparation stained with iron-hæmatoxylin alone, a few slides were counter-stained with Rubin S. Among 42 cases I found only two where I was inclined to count two fibers; in all other cases I was certain that only one fiber from each pole was attached to each V-shaped chromosome. My observations have therefore failed to add any facts supporting Baltzer's suggestion, which, however, future investigation may verify.

Baltzer ('09*a*) traced the heterochromosome in *Strongylocentrotus* and *Echinus* to the female pronucleus. Unfortunately I was not able to obtain suitable material for this purpose, but further investigation will probably reveal the same conditions as in *Echinus* and *Strongylocentrotus*.

Discussion.

Comparing the chromosomes with peculiar shape in *Echinus* and *Strongylocentrotus* with those in *Toxopneustes* we find that the hook-shaped chromosomes in *Echinus* and *Strongylocentrotus* have no exact equivalent in *Toxopneustes*. The two extremely long rod-shaped ones are found in the three species. The V-shaped chromosomes in *Toxopneustes* are very similar to the horseshoe-shaped chromosomes of *Echinus* in respect to their formation and the equal length of their two arms. They differ from the *Echinus* chromosomes as already mentioned in their length and slenderness. As in *Echinus* we are not able to distinguish a particular one of these three V-shaped chromosomes as a heterochromosome.

The discovery of individuality of form among the chromosomes in echinoids is a very valuable factor in support of Boveri's "Individualitätstheorie" of the chromosomes. One also welcomes every such means of distinguishing parental chromosomes in cross-fertilized eggs. *Toxopneustes* for instance has been used for cross-fertilization (Tennent, '07 and '10). These chromosomes which show marked individuality of form will be of special value in cases of cross-fertilized eggs, where, as shown by Herbst ('09) and Baltzer ('09*b*), the chromosomes of one parent are almost entirely eliminated during the first segmentation divisions.

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ON THE INHERITANCE OF COLOR IN THE AMERICAN HARNESS HORSE.

A. H. STURTEVANT, JR.

In a study of the English thoroughbred horse C. C. Hurst[1] has shown that chestnut is recessive to bay and brown. He supposes that the presence of black in the coat is the dominant character. Now black, gray and most roan horses also have black in their coats, but 95 per cent. of the English thoroughbreds are bay, brown or chestnut, so that Hurst was unable to verify his supposition. The American trotting and pacing horse, however, a close relative of the English thoroughbred, exhibits colors in proportions much more favorable for an investigation of this kind. These proportions are about as follows: bay, 53 per cent.; black, 13 per cent.; brown, 15 per cent.; chestnut, 14 per cent.; gray, 3 per cent.; roan, 2 per cent.; dun, .1 per cent.

Perhaps before going further it will be well to give a brief discussion of these colors. According to Miss F. M. Durham, as quoted by W. Bateson,[2] there are three pigments, yellow, black and chocolate, concerned in the color of horses, as in mice, rabbits and other animals. Chestnuts have the yellow pigment alone.[3] Bays have both yellow and black pigments, and browns are only very dark bays, shading into the self-colored blacks on the other extreme. Grays have black hairs mixed with white ones, usually in a dapple pattern. Roans are of at least three types. The most common are the bay, red or strawberry roans, which have yellow-black hairs intimately mixed with white ones. The black, blue or gray roans appear to differ from grays chiefly in that their black and white hairs are more intimately mixed. The chestnut roans have yellow and white hairs. As will appear later the fact that there is no black in this class introduces a possible source of error into my calculations. However, these chestnut roans are rare, forming something less than 10 per cent of the total number of roans seen on the streets of New York, and nearly all of those seen are heavy draught horses, so that I feel sure they are very rare indeed among blooded trotters. The official records do not distinguish between these three types of roans, but in the journals it is not rare to see a horse described as belonging to one of the two commoner classes, though I have never yet seen in them a reference to a chestnut roan. There are several types of duns, but, as appears in the list above, all are rare. In a few families duns seem to be dominant to bay, brown and black, and one was connected with gray, but beyond this I have found nothing about the color. Lastly there are a very few spotted trotters, but these are all poorly bred ones, with short pedigrees, and I have done nothing with them.

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[1] *Proc. Royal Soc.*, Vol. 77, B., 1906, p. 388.

[2] "Mendel's Principles of Heredity," p. 125.

[3] According to Bateson some chestnuts are really chocolates, but these are like the yellows in having no black.

I have tried to show here that Hurst's discovery of the dominance of bay and brown to chestnut holds good for the American harness horse, and that gray, black and roan, or all other colors containing black, are also dominant to chestnut. In order to avoid confusion I shall call this dominant factor for the presence of black Hurst's factor. Apparently all trotters have the factor for chestnut, which I shall represent by C. This factor is hypostatic to all the others here mentioned. The factor next highest in the scale is that for black, or Hurst's factor, H, its absence being h. Next higher is that for bay, B, its absence being b. At the top stand the gray and roan factors, G and R. Now most horses have neither of these last two, and are therefore $ggrr$. A chestnut will always be Chh, but may have any combination of the other factors and their absences, since they produce no visible effect in the absence of Hurst's factor. Self blacks are $CHHbb$ or $CHhbb$, since bay is epistatic to self black. Bays have one or two C's, H's and B's. Grays have C, H and G, and roans have C, H and R. Whether these last two must have B or not is not clear. I shall discuss the three epistatic colors more fully and give my theory as to brown when I have presented the evidence as to Hurst's factor.

The chief authorities for the statistics and color pedigrees given here have been Wallace's "Year Book of Trotting and Pacing" and Wallace's "American Trotting Register," both of which are official records.

It is a recognized fact among the breeders of harness horses that certain stallions never produce chestnut foals. In *Wallace's Monthly* for February, 1880, there is an article by "Truth," in which he says: "I have learned that neither of the brothers [Volunteer and Sentinel] have ever sired a chestnut colt." W. H. Marrett, in the September, 1890, issue of the same paper, tells us that the two bay sires Volunteer and Electioneer never had chestnut foals. Both were by a bay sire (Rysdyk's Hambletonian, which appears in the first table below), one being from a bay mare, the other from a brown. In a sale catalogue issued in 1903 C. W. Williams says of the brown stallion Belsire: "His get are . . . bays, browns and blacks." This horse is a son of the Electioneer mentioned above and of a black mare whose sire was a black and dam bay. He is a full brother to the bay Chimes which appears in the table below, and to Bow Bells, bay, and St. Bel, black, both also probably homozygous.

I have found a good many sires homozygous for Hurst's factor. The small number of gray and roan sires in the table below is to be explained by the small number of those colors existing. It will be noticed that two of the number have one chestnut foal each recorded. Director's was found in an advertisement in a horse journal—obviously a poor authority, as the pedigree might easily have been false. That by Jay Bird is Cardenas, trotting record 2:26¼, from a chestnut mare. He is recorded as a chestnut by the "Year Book." But the "Year Book" does sometimes make mistakes in the matter of color. Among others I could mention is the case of the bay stallion Charley Wilkhurst, recorded as a gray gelding.[1] In this connection it is worth noting that Hurst found about 1 per cent. of exceptions recorded in his investigation, but was able to explain most of them by showing them to be probably mistakes.

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[1] See *The Horse Review* for December 12, 1905, p. 1424.

TABLE OF SIRES HOMOZYGOUS FOR HURST'S FACTOR.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Chimes[1]	bay	bay	black	15	1	4	0	0	0	20
Electioneer[2]	bay	bay	brown	39	2	9	0	2	0	52
Happy Medium	bay	bay	bay	59	0	4	0	4	2	69
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Director	black	brown	bay	15	35	19	1	2	2	74
Gambetta Wilkes[3]	black	brown	brown	12	12	6	0	0	0	30
Oro Wilkes	black	black	black	15	3	4	0	0	0	22
Sable Wilkes	black	bay	black	23	19	7	0	0	0	49
Dictator	brown	bay	black	12	7	5	0	0	0	24
George Wilkes	brown	bay	brown or roan	12	5	3	0	0	1	21
Prodigal	brown	bay	bay	94	0	10	0	3	0	116
Conductor	gray	bay	gray	3	1	5	0	15	0	24
Pilot Medium	gray	bay	gray	43	0	5	0	61	1	110
Eagle Bird	roan	roan	brown	15	1	4	0	0	8	28
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

[1] Half of these foals from mares by the chestnut Mambrino King.

[2] Twenty-nine of these chestnut mares.

[3] Eleven of these from chestnut mares.

I have found only 69 cases of chestnuts being mated together, but in all these the result was chestnut. They are from the following stallions:

Chestnut Sire.	Color.		Number of Chestnut Foals from Chestnut Mares.
	Sire.	Dam.	
Nutwood	bay	gray	10
Robert McGregor	bay	bay	7
Attorney	bay	?	6
Daniel Lambert	bay	?	5
Elyria	chestnut	black	4
Hinder Wilkes	bay	chestnut	4
Mambrino King	black	?	4
Six different sires with two each			12
Eleven sires with one each			11
Total			69

The finding of foals from heterozygous sires and chestnut mares is a very slow and laborious task, but I have found some from the following stallions:

Stallion.	Color.	Foals from Chestnut Mares.	
		Chestnuts.	Total Found of all Colors.
Alcyone	bay	3	7
Axtell	bay	8	10
Boreal	bay	6	8
Guy Wilkes	bay	7	8
Norval	bay	1	6
Onward	bay	8	20
Red Wilkes	bay	8	14
Strathmore	bay	3	6
Total bay sires	bay	44	79
Grattan	black	1	4
Simmons	black	5	7
Total black sires	black	6	11
Alcantara	brown	2	8
Allerton	brown	8	17
Total brown sires	brown	10	25
Alcryon	gray	2	2
Re-election	gray	3	4
Total gray sires	gray	5	6
Grand Total		65	121
Expectation		60½	121

Here again I have been handicapped in working with gray and roan by the small number of sires of those colors, and also by the fact that most of the best known of those existing seem to be homozygous. However, I have found some sires of those colors which throw a fair percentage of chestnut foals, as shown in the following table, which shows all known foals. Two blacks are also included.

Stallion.	Color.	Chestnut Foals.	Foals of All Colors.
Bellini	black	3	17
Mambrino Patchen	black	3	16
Alcryon	gray	9	30
Pilot, Jr.	gray	4	20
Jay Hawker[1]	roan	1	20
Roan Wilkes	roan	3	10
Tom Hal, Jr.[2]	roan	1	15

[1] The one chestnut from Jay Hawker is scarcely to be doubted, as he is Country Jay 2:07¼, world's champion trotter under saddle, and one of the most prominent race horses of the season of 1909.

[2] This one chestnut also is not a doubtful one. His name is in fact Chestnut Hal.

It seems to me that we have here sufficient proof that Hurst's original supposition is right—that the dominant character is the black in the coat. However his idea that the black, if present, exists on the fetlocks, seems to me to be unjustified, as Joe Patchen, a heterozygous black, has white feet, though the white does not reach as far up as the fetlock on the left hind one. Grattan, another heterozygous black, also has three white fetlocks. It is not at all rare to see pictures of bays or browns with one foot or more white as far up as the fetlock or further. Here are some examples: bays—Capo, both hinds; Moko, right hind; Ario Leyburn, left hind; Allerworthy, both lefts; Hail Cloud, right front; browns—Redlac, both hinds; The Harvester, left hind; Searchlight, both hinds. At least one of these (Moko) is homozygous.

In the case of the next factor, bay, a complication arises in regard to brown. As explained before the presence of bay is dominant to its absence and the color next below it in the scale is black. Brown is a color between these two, shading into both extremes. It seems that brown is usually a heterozygous color, represented by *CHBb*, but that bay also is quite often heterozygous, and that brown may occasionally be *either* of the homozygous types, *CHBB* or *CHbb*. This suggests the idea that the line between black and bay should be drawn somewhere near the black limit of brown. The obvious result of this complication is the creation of considerable confusion in the numerical proportions of the two colors. It is evident that, except for this complication and the appearance of some chestnuts, bay will act as though it were an ordinary dominant to black.

Below is a table showing twelve sires homozygous for the bay factor. Six of them are bays, and one is the only brown certainly known to be homozygous for this factor. Two are chestnuts, and therefore lack Hurst's factor. The other three are a gray and two roans, and it will be noted that all three of them appeared in the table of sires homozygous for Hurst's factor. They are not bays because they also bear other factors, as I shall explain later. Of the bays two are homozygous and four are heterozygous for Hurst's factor. The single black from Robert McGregor is Bobby Good, pacer, 2:11¼, out of a daughter of Ashland Wilkes.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Ashland Wilkes	bay	bay	?	90	0	0	19	5	0	114
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Happy Medium.	bay	bay	brown	59	0	4	0	4	2	69
Onward	bay	brown	bay	85	0	7	24	1	0	117
Red Wilkes	bay	brown	?	92	0	7	22	3	0	124
Sphinx	bay	bay	chestnut	30	0	5	9	4	0	48
Prodigal	brown	bay	bay	94	0	19	0	3	0	116
Axworthy	chestnut	bay	bay	33	0	2	32	1	4	72
Robert McGregor	chestnut	bay	bay	42	1	1	30	2	1	77
Pilot Medium	gray	bay	gray	49	0	5	0	61	1	116
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

The mating together of blacks should produce only blacks and a few chestnuts, since chestnut is the only color hypostatic to black. However it does produce some browns and is recorded as producing occasional bays. The bays so far found are: Kipling 2:21¼, by Gambetta Wilkes ex Margaret W., and Gipsey Bel 2:30, by St. Bel ex Gipsey A. Now there is some reason to doubt the color of St. Bel. His dam was the black Beautiful Bells, but his sire was the bay Electioneer, and he is the only black among the eleven foals by Electioneer from black mares that I have found. Moreover, counting St. Bel, I have found only two black foals out of a total of 52 from Electioneer. Since Electioneer was homozygous for Hurst's factor this small proportion cannot be partly explained by supposing that more of the 52 would have been black had they had that factor. Neither is it possible to suppose that the small proportion is due to suppression by the gray or roan factor, since the 52 include only two grays and no roans. It looks very much as though Electioneer were homozygous for the bay factor. St. Bel died young, leaving few foals, so that I have been unable to get much data about his descendants. As to the other apparent exception, Kipling, I shall only call attention to the fact that neither he nor his dam are very well known. The following table shows foals from two black parents.

The case of foals from heterozygous sires and black mares is

Black Sire.	Foals from Black Mares.			
	Bays.	Blacks.	Browns.	Chestnuts.
Bellini	0	2	0	1
Gambetta Wilkes	1	3	0	0
Grattan	0	3	0	0
Patchen Wilkes	0	8	0	0
Sable Wilkes	0	7	1	0
Simmons	0	3	0	0
Nine others	1	8	3	0
Total	2	34	4	1

the one where the uncertainty about brown causes the most trouble. Since the presence of Hurst's factor is necessary before any of the three colors in question can appear I have left out the chestnut foals in the following table of foals from black mares.

Stallion	Color	Foals from Black Mares.		
		Bays.	Blacks.	Browns.
Axtell	bay	1	4	3
Guy Wilkes	bay	1	7	3
Alcantara	brown	1	3	0
Allerton	brown	0	3	3
Nutwood	chestnut	2	3	0
Total		5	20	9

The numbers in the above table are small, and, as in the similar case with Hurst's factor, I have supplemented it with one giving all known foals, chestnuts being again left out.

Stallion	Color	Foals				
		Bays	Blacks	Browns	Grays.	Roans.
Bow Bells	bay	8	2	0	0	0
Directum Kelly	bay	7	2	2	0	0
McKinney	bay	14	4	8	0	2
Baron Wilkes	brown	44	10	28	0	1
Brown Hal	brown	34	10	15	2	4
Dictator	brown	12	7	5	0	0
George Wilkes	brown	12	5	3	0	1
Highwood	brown	24	5	8	0	0
American Star	chestnut	6	2	1	1	0
Mambrino King	chestnut	4	3	1	0	0
The Earl	chestnut	2	1	1	0	0
Conductor	gray	3	1	3	8	0
Pilot, Jr.	gray	3	2	1	10	0
Eagle Bird	roan	15	1	4	0	8

Now to turn to the gray factor. In the first place, I make no claims that all gray is epistatic to the four usual colors. Perhaps it will be best to take up the grays by families, and I will first treat of those in which it is epistatic, and then of the one in which it seems not to be.

Most of the high-bred grays of to-day go back to Pilot, Jr., through an unbroken line of grays. This horse was a gray, son of a black sire and of a mare of untraced breeding whose color I have been unable to find. The gray sires in the next table all get their gray from him. This table includes all known foals except chestnuts, these being omitted for the same reason as in the last case. Since gray is an unpopular color it is safe to say that nearly all these foals were from recessive (*gg*) mares. I have so far found only one case of grays being mated together, and, since the produce of that mating was never heard from after racing, I know of no horse homozygous for the gray factor, *G*.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Bayard	gray	?	4	5
Pilot, Jr.	black	?	5	10
Pilot Medium	bay	gray	55	61
Re-election	bay	gray	12	15
Total			76	91

The following sires are all sons of gray members of the Pilot, Jr., family. Several of them have gray foals in this table, but all of these are from gray mares.

Stallion.	Color.	Foals				
		Bays.	Blacks.	Browns.	Grays.	Roans.
Lord Russell	bay	16	0	0	1	0
Peter the Great	bay	24	1	2	2	0
Darknight	black	5	2	2	0	0
Electricity	brown	10	1	7	1	0
Expedition	brown	46	3	10	5	0
Highwood	brown	24	5	8	0	0
Mambrino Russell	chestnut	6	0	3	0	0
Nutwood	chestnut	70	9	2	3	0

There can be little doubt that in the Pilot, Jr., family gray is an ordinary dominant, and there are other families where it seems to be, though there is not as much evidence. One of these goes back to the mare Sontag Mohawk, and through her probably to imported Messenger, the foundation of the breed of American harness horses. Another goes to the mare Bashaw Belle, daughter of Young Bashaw, gray. Of the three horses below, Conductor is a son of Sontag Mohawk, Manager is a grandson of Bashaw Belle, and Alcryon is out of Lady Blanche, daughter of Privateer, both grays.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Alcryon . .	bay	gray	14	7
Conductor .	bay	gray	7	8
Manager .	chestnut	gray	11	12
Total .			32	27

Eros and Walnut Hall, a brother and son, respectively, of Conductor, and both browns, have no gray foals among the 35 I have found.

There is one family in which gray appears not to be epistatic. The first member of the family that I know of is General Wilkes, gray, son of George Wilkes (an ordinary brown which appears in two of the tables already given and has nine different sons in them) and of a gray mare. This stallion had some gray foals, of whose descendants I have found no record. But he had two sons not gray which have produced many gray foals. They are Dispute, black, and Bobby Burns, bay. I am not sure of the color of the dams of any of Dipute's gray foals, but in the case of Bobby Burns some of them are from bay mares. Dispute's maternal color pedigree I do not know, but Bobby Burns is from Dixie, a bay daughter of the brown Dictator appearing in some of the first tables in this paper. The colors of all foals found from Dispute and Bobby Burns are:

Dispute: bay, 3; black, 2; gray, 4; all, 9.

Bobby Burns: bay, 40; black, 10; brown, 10; chestnut, 3; gray, 43; all, 106.

This is certainly a different kind of gray from the others just described, but I have not enough data to try to explain it.[1]

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[1] In connection with the bay and gray factors I may quote the following from the pen of Professor Karl Pearson ("The Law of Ancestral Heredity," *Biometrika*, 1903, vol. 2, p. 214), though it was written about the English thoroughbred: "If black or gray coat-colour in horses were 'recessive,' when two blacks were mated we should expect only black offspring, but black can disappear for a generation or even two and then reappear. Or, take a case like that of a gray horse Viscount,

The last character I have to deal with is roan. This, like gray, is epistatic to the four usual colors in most families, but may not be in all.

Many of the roans of to-day go back to the old roan race-mare Lady Franklin, through her daughter Lady Frank and grandson Jay Bird, both roans. Jay Bird sired Eagle Bird, Jay Hawker, Allerton and Jackdaw, and Jay Hawker sired Jay McGregor. The following table shows all foals but chestnuts.

Stallion.	Color.	Foals.					
		Bays.	Blacks.	Browns	Grays.	Roans.	All not Roan.
Eagle Bird	roan	15	1	4	0	8	20
Jay Bird	roan	54	0	15	3	63	72
Jay Hawker	roan	6	0	0	0	13	6
Total		75	1	19	3	84	98
Jay McGregor	bay	11	1	5	0	0	17
Allerton	brown	32	8	20	2	1	62
Jackdaw	brown	9	3	12	1	0	25

Allerton's roan foal is from a roan daughter of Jay Bird.

Another family goes back to Laura Fair, roan, through her roan granddaughter Spanish Maiden. This last mare produced 1 bay and 3 roans, including the sire Margrave. Tom Hal, Jr., founded another family of roans, and another goes to the roan mare Tilla, which had 4 bay foals, 1 brown and 3 roans, the latter including Fred S. Wilkes. The Brown Hal appearing in the table below is a son of Tom Hal, Jr. Chestnut foals are omitted as before.

Stallion.	Color.	Foals.	
		Not Chestnut or Roan.	Roan.
Fred S. Wilkes	roan	13	8
Margrave	roan	35	30
Tom Hal, Jr.	roan	7	7
Total		55	45
Brown Hal	brown	61	4

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where gray remained dominant for three generations only to disappear before the chestnut of the mare Blue Stocking in the Viscount and Blue Stocking filly Miss Johanna!" Just what that passage was intended to mean is a problem which I have not yet solved. What do blacks produce when mated together, and what has that to do with skipping a generation or so? And if a recessive cannot skip what can? Certainly not a dominant. What is to prevent us from supposing Viscount a heterozygote?

The roan and also the two gray foals of Brown Hal probably get their color from their dams, since in all cases these were daughters of roan or gray sires. As to the kind of roans these are I can only say that Margrave and one of his foals, and one of the foals of Tom Hal, Jr., are all red roans.

I have found six roan foals which had neither parent roan. One was from a dun mare. One was from two bays and another was from a chestnut and a bay, the chestnut being Robert McGregor, which almost certainly carried no gray factor. Another was from a bay sire and a chestnut dam. The other two each had a gray parent, and at least one of them was a black roan. Now only six roans produced by the thousands of cases of mating together horses not roan is a very small percentage. It is to be noticed that these six are not closely related. Even the two grays concerned are one Pilot, Jr., and one Sontag Mohawk. It seems to me probable that red roan at least is an ordinary dominant, and that all but one of the above cases are mistakes or exceptions. It is quite possible that all horses having the factor *R* are roan, the type of roan depending upon the color the horse would have been if he had not had that factor. This, if correct, explains away the difficulty presented in the next paragraph in so far as roan is concerned.

The relation between gray and roan is not clear. It seems probable that some of the *black* roans may be connected with gray, but an examination of the tables given above will convince one that, in general, the two colors are quite distinct. I have found only two instances of the mating together of grays and roans, and in both the result was gray. Of course much more evidence would be needed in order to find out how they act toward each other. The relation between these two factors and the bay factor is also not quite clear. It is evident that the presence of either can conceal bay, but whether or not either can appear in the absence of the bay factor is not certain. I am inclined to think that they can. If gray cannot we have an explanation of the gray foals from the black Dispute, but get no help in the much harder problem concerning his bay half-brother, Bobby Burns. One would expect to find some chestnuts carrying gray or roan factors (if the *ChhR* horses are chestnut roans there should be no chestnuts carrying the roan factor). With one possible exception in the case of each color I have found no such case. How unsatisfactory these two cases are will appear from the color pedigrees of the horses concerned.

Molly Morton, gray.	Banker Rothschild, brown.	Rothschild.
		Pilot Anna, gray.
	Lady Forrester, chestnut.	Royal George, chestnut.
		Belle of Saratoga, brown.
North Wind, roan.	Young Clay Pilot, bay.	Clay Pilot, bay.
		?
	Lola M., chestnut.	Roy Executor, brown.
		Light Dale, chestnut.

SUMMARY.

This study of the pedigrees of blooded trotters indicates that the color of such horses is usually controlled by five factors, as follows. First, a factor for chestnut, *C*, present in all the horses studied. Second, a factor for black, Hurst's factor, *H*, epistatic to the factor *C*, and hypostatic to the three following. Third, a factor for bay, *B*. Fourth, a factor for roan, *R*. Fifth, a factor for gray, *G*. *R* or *G* inhibits *B* if it is present, but whether they depend upon its presence for their own appearance or not is not clear.

COLUMBIA UNIVERSITY,
June, 1910.

THE MARSUPIUM OF THE ANODONTINÆ.

In the June number of the BIOLOGICAL BULLETIN (p. 31), G. Lefevre and W. C. Curtis have published a paper on "the Marsupium of the Unionidæ," in which they say that the lateral (secondary) water tubes cut off from the original (primary) water tubes in the marsupium of the Anodontinæ, described by myself in *Nautilus*, February, 1910, are *not present*. In order to show this, they publish three figures of horizontal sections through the marsupia of three species of Anodontinæ.

To obviate misunderstanding, I want to point out, that, in two of the figures referred to, these secondary water tubes ARE PRESENT, most *beautifully* and *typically* in Fig. 1. In Fig. 3 traces of them are observable, while in Fig. 2 they are *not yet* developed. These tubes are *not* blood vessels, as might be believed after superficial investigation.

For the rest, I cannot go into detail, but must refer to my future publication (illustrated by microphotographs) in the *Memoirs of the Carnegie Museum*, where additional facts will be published.

A. E. ORTMANN.

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BIOLOGICAL BULLETIN

ACCESSORY CHROMOSOMES IN MAN.

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Since the publication of my papers on the spermatogenesis of the guinea and of the chicken respectively (Guyer '09*a*, '09*b*) in which was recorded the finding of a chromosome or chromosome complex comparable to the "odd," "accessory" or "X-element," described so frequently of late as occurring in a wide range of the Arthropoda, particularly the Tracheata, I have examined material from other vertebrates and can at present record its presence in the rat, its probable occurrence in the pigeon (although this will require some further corroboration), and its conspicuous occurrence in man. Inasmuch as the material for its study in the rat is in the hands of a student for further investigation, I shall confine myself in this paper to a description of the two accessory chromosomes as found in man, together with other features of human spermatogenesis.

For my studies on man I have been fortunate in being able to obtain exceptionally good material through the courtesy of my colleague, Dr. Paul. G Woolley. The subject from which the testicular material was secured was a negro thirty years of age who had died suddenly from the rupture of an aortic aneurism (Case no. 156143, Pathological Records, the Cincinnati City Hospital). A testis was removed within between an hour and an hour and a half after death while the body was still warm and slices were placed immediately into Gilson's and into Bouin's fluids.

The mounted sections were from five to twelve microns thick. Most of them were stained in Heidenhain's iron-hæmatoxylin and counter-stained with Congo red or acid fuchsin, although Delafield's hæmatoxylin was used with some.

An abundance of cell divisions were found to have been in progress at the time of death. In a given field of the microscope, in a favorable region, it was not unusual to observe as high as six or seven cells in various phases of division. As many as five or six of such areas might exist in a single section, although it was more usual to find only one or two. The material was very uneven in that slides would be found in which section after section showed division stages, while in others divisions were scarce. These facts indicate that there were proliferating and resting zones in the testis. The stages found in most abundance were the metaphases and late prophases of the primary spermatocytes. It was a comparatively simple matter to find spindles on which the ordinary chromosomes were in metaphase with the two accessories, closely associated, well removed toward, or at, one pole (Figs. 6, 7, 8 and 9).

In the literature of the subject much confusion prevails regarding the number of chromosomes characteristic of man. There is wide disagreement in the counts of different observers and there seems to have been a great dearth of material showing division stages. Most of the enumerations are based on observations of from two to eighteen cells and these often in questionable stages of preservation. The great difficulty apparently has been to secure material which was sufficiently fresh or which was not diseased tissue that is notoriously irregular as regards karyokinetic phenomena.

As early as 1881 Flemming discussed mitosis in the case of man illustrating it with some six figures (Taf. 3, Figs. 11–16) of which Fig. 16 is from leucocytes of leucemic blood, the others, from the corneal epithelium of two different subjects from each of whom an eye had been removed because of affection of the bulbus. Although at this time he made no definite record of the number of chromosomes, his drawings show them to be considerably in excess of sixteen, the number later announced by Bardeleben ('92).

Writing several years later, however, in response to the 1892 paper of Bardeleben, Flemming ('97), from a reëxamination of his old material, gives the number of chromosomes as twenty-four. He cites the papers of Hansemann ('91, '93) as the earliest attempts known to him to make a count of the chromosomes of man. But since Hansemann records eighteen in one case, twenty-four in another, and forty in a third, the latter apparently estimated from the spireme stage, and inasmuch as he himself admits that his count was very uncertain, concluding with the statement that, "die Zahl sicher höher als 24 sei," we may fairly disregard it, I think, in the light of modern technique. In this second paper Flemming ('97) states that his count is based on only four cell-divisions in which the chromosomes had just split preparatory to separation. His exact statement of his observations is as follows: "Es gelang das zwar bei keinen ganz sicher, aber bei zweien der vier darin enthaltenen Mitosen doch annähernd; es scheinen in beiden Fällen 24 Doppelchromosomen zu sein. Bei beiden sind es jedenfalls mehr als 22 und, wie ich sagen zu können glaube, weniger als 28; an einiger Stellen decken sie sich so, dasz eine exacte Zahlung mir unmöglich wird." Flemming's material had been fixed in one sixth per cent. chromic acid and stained with safranin.

Bardeleben has published three papers ('92, '97, '98) on the spermatogenesis of mammals including man in which he comes to the conclusion that the number of chromosomes in the spermatogonia and spermatocytes of man are sixteen and eight respectively, and in his later papers he sets down four as the number that ultimately reaches the spermatids. That is, there is in successive divisions a reduction in numbers from sixteen to eight and then from eight to four. This is much the condition that I have found prevailing in birds (Guyer, '02, '09).

Wilcox ('00) studied sections from a testis which had been removed from a man fifty-four years old, in an operation for hernia. Although scrotal swelling had existed for a year previous to the operation the testis seemed to be normal in size and appearance. He reports that, "In my material the number seemed to be eighteen, the different counts resulting in figures ranging from fifteen to nineteen." He remarks however upon the striking absence of karyokinetic stages, so that his observations were based upon a very limited number of divisions. Because of this lack of favorable stages he says: "The whole organ was, therefore, sectioned and in the great number of sections thus obtained, not more than twenty cells were found in mitotic condition."

Wilcox continues: "The few cases of mitosis observed were in spermatocytes of the first order. One could easily distinguish spermatogonia, spermatocytes of the first and second order, spermatids, and numerous nearly mature spermatozoa. The number of the latter to be seen was very large and precludes the assumption that the testis was functionally impaired by age or by hernia. In the opinion of the writer, this condition merely indicates that all the various processes in the spermatogenetic series are not necessarily to be observed as taking place at the same time. I can see no reason why there might not become established in the testis periods of cellular activity alternating with periods of cellular rest."

Unfortunately Wilcox gives no drawings with his paper nor does he state definitely whether he regards the eighteen chromosomes seen in the spermatocytes of the first order as the reduced number or not. He does remark, however, that, "in many cases they were plainly arranged in the tetrad or ring formation which has been observed in a pretty general variety of investigated species," consequently the inference would be that a synapsis had occurred and that one might expect to find in the neighborhood of thirty-six as the somatic number.

The latest investigation on the number of chromosomes in man with which I am acquainted is that of Duesberg ('06). He reviews the work of Hansemann, von Bardeleben, and Flemming and on the strength of his own observations concludes that Flemming's count of twenty-four is correct. The excessive number found by Hansemann he would account for on the basis of the abnormal increase in the number of chromosomes which is likely to occur in pathological tissues. In the case of Bardeleben he is inclined to believe that very thin sections (three microns) are responsible for the smallness of the count since he regards it as probable that part of the cell had been cut away.

The tissue upon which Duesberg worked had been fixed in Flemming's or in Hermann's fluid and stained by the iron-hæmatoxylin method. However, the number, twenty-four, which he records for man was not determined by direct count but was inferred from the fact that he found two or three clear cases of twelve chromosomes in the primary spermatocytes. That his finding of twelve in the primary spermatocytes was correct is borne out by my observations but he is not justified, in consequence, in stating that there must be twenty-four in spermatogonial or somatic cell-divisions. My material shows that two of the twelve chromosomes are the univalent accessories, and a clear count of favorable spermatogonial chromosomes reveals only twenty-two. This means in all probability that of the twelve chromosomes of the primary spermatocyte, ten are bivalent and two accessories. Although Duesberg examined the spermatogonial chromosomes he states that he was unable to count them exactly beyond determining that, contrary to the opinion of Von Bardeleben, there were clearly more than sixteen. He states further (p. 477) that, "Je n'ai pas pu les compter exactement, tant à cause de la petitesse des cellules que du nombre assez élevé des chromosomes, mais dans quelques cas favorables où leur numération a pu être entreprise, j'ai obtenu des résultats très voisins de 24, jamais supérieurs à ce nombre dans les cellules normales." And in conclusion he says: "Il résulte de là que le nombre des chromosomes est certainement à mon avis, de 12 dans les spermatocytes et par conséquent de 24 dans les spermatogonies et les cellules somatiques. C'est la confirmation de l'opinion de Flemming."

In the general scheme of the spermatogenesis of man there appears to be nothing unique. One can readily recognize the usual four generations of germ cells; viz., spermatogonia, primary spermatocytes (or spermatocytes of the first order), secondary spermatocytes (or spermatocytes of the second order), and lastly spermatids which transform directly into the spermatozoa. An abundance of easily identified Sertoli or nurse cells are in evidence. Occasional centrosomes were observed in suitably stained preparations but I have not pictured any in my drawings because the preparations from which the latter were made were all so strongly decolorized that the stain had evidently completely disappeared from any centrosomes which might have been present.

The matter of counting the spermatogonial chromosomes, it must be admitted, is one of great difficulty. In the late prophase or equatorial plate stage, the only time at which a count is possible, they lie for the most part in an irregular band around a central clearer area. In the vast majority of cases only a deeply stained mass of small contiguous or overlapping chromosomes is visible in this band and an accurate count is out of the question although one can frequently determine that there are over twenty. In several instances, however, in which the positions of the chromosomes and the degree of the staining were favorable, twenty-two distinct chromosomes, never more, were visible.

There is considerable range in size among the individual chromosomes of the spermatogonia as well as observable differences of form. Most of them were rod-like or oval in shape although some were more nearly spherical. In several though by no means all instances two chromosomes, closely associated, were seen lying at some distance away from the main band, out in the cytoplasm. Taking into account this isolation, the rounded shape of these chromosomes and their relative sizes, it seems very probable that they are the two accessory chromosomes which do not manifest their presence for a certainty until the next division. It will be observed that one is somewhat smaller than the other. This condition obtains also between the two chromatin nucleoli of subsequent stages as well as between the accessory chromosomes whereever they can be identified, and one is led in consequence to strongly suspect that they are all one and the same thing. This inference is all the more justifiable when the relation between the chromatin nucleoli and the accessory chromosomes in some of the lower forms is recalled.

Fig. 2 represents a nucleus of the primary spermatocyte in the spireme stage which shows the two chromatin nucleoli in question. In deeply stained specimens these nucleoli, especially the smaller one, are not always evident but in preparations stained by the iron-hæmatoxylin method and then almost entirely decolorized, even as regards the ordinary chromatin of the spireme they are usually conspicuously visible. It should be mentioned that occasionally other small nucleolus-like granules were observable but since there was no constancy in their presence, size or relationship, I have felt justified in ignoring them in the present discussion. Fig. 3 represents the spireme in the contraction phase which is not very pronounced in man. It will be observred that the two characteristic chromatin nucleoli still persist.

The primary spermatocytes when ready for division, as has already been stated, reveal twelve chromosomes in late prophase or early metaphase (Figs. 4, 5). In Fig. 4 the two accessories are seen at a glance and the remaining chromosomes, judging from their increased size and changed form, are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original twenty-two chromosomes twenty have paired to form the ten bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. In Fig. 4 it is not evident just which two are the accessories although twelve chromosomes are present.

It is obvious from the the figures (Figs. 4–9) that there is considerable difference in the size of the various chromosomes of the primary spermatocyte. Although the attempt was made it was not found possible to always identify the individual chromosomes. They grade down in size from some three or four large ones to two or three small ones but the fluctuations in size, probably due for the most part to differences in the effects of fixation together with different degrees of extraction of the stain, were too great to render identification sure. In very strongly decolorized sections, especially when counterstained with Congo red, one large chromosome in particular frequently exhibited a tetrad-like formation, while the other large ones at times showed more or less definite indications of lobing. In some cases this was sufficiently marked to interfere with accurate counting. In a very few instances, so few I think as to be practically negligible, there appeared to be fourteen instead of the customary twelve chromosomes, but the extra chromosomes always took the form of a tiny pair which I am inclined to think had become split off from one of the ordinary tetrads or which had through some chance never entered into the proper tetrad formation. They were always united by linin-like strands to one or two of the larger chromosomes.

Figs. 6, 7, 8 and 9 show the two accessories in characteristic positions. Side by side, they always pass entire, considerably in advance of the divided ordinary chromosomes, toward one pole.

Of the ordinary daughter chromosomes of this first spermatocytic division, a pair of small elongated ones not infrequently are the first to emerge from the general equatorial mass as shown in Fig. 7. One is led to suspect that they may possibly be comparable to the small pair of chromosomes found so constantly in certain of the Tracheata although the evidence is not sufficiently decisive to make this an established fact.

It is inferred that the division of the primary spermatocyte is the reducing division, not simply because such a division ordinarily occurs at this stage, but from the fact that the chromosomes after divergence (Figs. 10, 11) when compared with corresponding divisions of the secondary spermatocytes are seen to resume more the elongate, rod-like appearance that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole here while they are halved in the next division.

It is evident from the foregoing that as regards chromatin content the result of the division of the primary spermatocyte is the production of two dissimilar cells, one of which receives ten, the other, twelve chromosomes. Fig. 10 is a drawing of one end of a late anaphase of such a division showing twelve chromosomes (10 plus 2 accessory). Fig. 11, in which only ten chromosomes are visible, was drawn from what is probably the reverse end of a somewhat later anaphase than that shown in Fig. 10. It is just possible that it is a prophase of division in a secondary spermatocyte where univalent chromosomes come to the equator, but if so it is the exception rather than the rule, as the secondary spermatocytes ordinarily divide according to a different scheme. In any event the drawing serves to illustrate the fact that some daughter cells of the primary spermatocytes have twelve chromosomes and some only ten.

In places both primary and secondary spermatocytes were found dividing in the same field and one is led to conclude that either there was no intervening period of rest between the two divisions or that it was a very brief one. In other instances, however, undoubted resting stages of secondary spermatocyte nuclei were seen in abundance. Approximately half of them showed, under proper decolorization, two chromatin nucleoli of which one was somewhat smaller than the other.

While at the conclusion of the divisions of the primary spermatocytes ten and twelve chromosomes respectively were delivered to the pairs of daughter cells, nevertheless, when the latter as secondary spermatocytes become ready for division, half of them show five and the remainder seven chromosomes. A second pairing of the ordinary chromosomes has evidently occurred, so that there are five bivalent chromosomes in each type of cell and the additional two accessories in the one type. Fig. 12 is a drawing of two contiguous secondary spermatocytes; the one shows five bivalent chromosomes in late prophase, the other more than five chromosomes in metaphase. These two cells are undoubtedly the two daughter cells of the same primary spermatocyte. Fig. 13 shows one daughter cell containing a group of seven chromosomes and the other a late anaphase of division which shows at one end five chromosomes. The number of chromosomes at the opposite pole of the second cell should of course be five although because of the dense massing it could not be positively determined. Fig. 14 represents a late anaphase of division in a secondary spermatocyte which manifestly had had seven chromosomes in metaphase.

Both accessory chromosomes divide in this second spermatocytic division period so that each resulting spermatid receives seven chromosomes (Fig. 16). Fig. 15 represents an anaphase of division in a secondary spermatocyte showing still at the equator of the spindle a lagging chromatic mass. Such a condition was found in several instances and while I believe it to be the two accessory chromosomes which happened merely to be unfavorably placed for observation, I could not positively identify it as such. From the relative positions of the chromosomes as seen in Fig. 16 one would infer that the two sets of accessories were the last to have passed from the equator to the poles of the spindle. Moreover, such a lagging of the accessory in this division was observed in both the guinea and the chicken (Guyer, '09).

It should be mentioned that occasional division stages were visible which, judging from the smallness of the cell and the size and shape of the chromosomes, looked as if they might be secondary spermatocytes preparing to divide with the univalent type (ten or twelve) of chromosome. It is possible, for instance, that

Fig. 11 represents a prophase of the secondary rather than an anaphase of the primary division although I am inclined to think it is the latter. If such simple divisions do take place, however, they are certainly scarce in the material which I have examined so far.

From the foregoing evidence it is manifest that there are in all two distinct groups of spermatids equal in number; namely, those which have received five and those which have received seven chromosomes. These chromosomes soon lose their visible identity and the spermatids are apparently all alike except for the significant fact that approximately half of them, in such preparations as have been stained by the iron-hæmatoxylin method and then all but entirely decolorized show two chromatin nucleoli. It would seem probable that these nucleoli stand in direct genetic continuity with the two eccentric chromosomes seen in the spermatogonia and the two chromatin nucleoli and the accessory chromosomes of the spermatocytes. Fig. 18 represents two contiguous spermatids, one of which shows no nucleoli, the other, two. Comparison with Fig. 19 shows the relative conditions of size between the nucleoli of the spermatid and those of a primary spermatocyte.

As to the meaning of the second conjugation there seems to be at present no clew. I have commented on it briefly in a former paper ('09*a*, p. 509). It is not peculiar to man for I have observed it also in the pigeon ('02, '03), the guinea ('09*a*) and the rooster ('09*b*). Undoubtedly Bardeleben ('97, '98) still earlier saw the same phenomenon in man, for although my results do not agree numerically with his count of sixteen, eight and four respectively, evidently, from the relative proportions in his counts, he had come upon this second curious numerical reduction.

Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia or in anaphases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that is characteristic of the bivalent types (compare Figs. 1, 10 and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively.

Inasmuch as the spermatids transform directly into spermatozoa it follows that there must be be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes. Thus the conditions in man appear to be very much the same that Wilson ('09) describes for *Syromastes*, one of the squash-bug family (Coreidæ).

Numerous examples of such dimorphism of the spermatozoa have been recorded in various invertebrates, particularly in insects, arachnids and myriapods, and it has been clearly demonstrated that eggs fertilized by spermatozoa which possess this accessory chromosome or chromosome group (there may be one, two, three or even four separate chromatic bodies, depending upon the species) develop into females, those fertilized by spermatozoa which do not possess it, develop into males. Hence the accessory has come to be regarded by some of our most careful and experienced workers as an actual sex determinant. In any event it is obviously associated with the determination of sex either as cause or effect. In the light of numerous recent researches both on plants and animals the idea has rapidly gained ground that sex arises not as was long believed, as a response of the developing organism to stimuli from without but that under normal conditions at least, it is automatically determined by some internal physiological mechanism.

Inasmuch as this intricate matter has been repeatedly and exhaustively discussed pro and con during the past ten years, it is unnecessary for me to enter into a review of the subject anew. For the general reader who may not have kept in touch with the current literature of the subject, two excellent critiques are now available in the recent papers of Wilson ('09*a*) and Morgan ('10). In these papers one will also find thoroughgoing discussions of the subtle problem as to whether, assuming that the accessories are sex determinants, the matter of sex determination is to be regarded as a qualitative process effected by some inherent peculiarity of the accessory chromosome, or whether the relation of such a chromosome or group of chromosomes to sex is merely a quantitative one, the female type resulting when a greater amount of active chromatin is present. Extensive bibliographies will be found in the recent papers of Wilson ('05, '06, '09), Payne ('09), Morse ('09) and Morgan ('10).

In conclusion I wish merely to point out that as regards accessory chromosomes, conditions prevail among vertebrates (guinea, chicken, rat, man, etc.) similar to those found among numerous Tracheata (and probably certain other invertebrates) where the accessories are undoubtedly associated in some way with the phenomena of sexuality. In *Syromastes* (Wilson, '09*b*), which seems to parallel most nearly the condition found in man, half of the spermatids were found to possess two more chromosomes than the remainder. It was predicted by Wilson that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male. Later the facts were found to be in exact accord with his prediction, the somatic cells of the female containing twenty-four, of the male twenty-two chromosomes. Similar verifications have been made in other tracheate forms.

In the light of these facts we should expect the somatic cells of man to contain twenty-two, and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above.

Summary.

1. Twenty-two chromosomes differing considerably in size occur in all spermatogonia in which a definite count could be made. In a few instances two, apparently the two accessory chromosomes, were seen considerably to one side of the main mass of chromosomes, surrounded by a small clear court of cytoplasm.

2. Twelve chromosomes appear for division in the primary spermatocyte, of which ten are evidently bivalent and two accessories.

3. The two accessory chromosomes pass undivided to one pole of the spindle considerably in advance of the other chromosomes, with the result that half of the daughter cells in this division receive twelve, and half, only ten univalent chromosomes. This is evidently the reduction division.

4. The ten univalent chromosomes which passed to the one secondary spermatocyte unite again in pairs, at least in the majority of cases, to form five bivalent chromosomes which appear at the equator of the spindle when the cell is ready for division. The division here is presumably an equation and not a second reduction division, judging from the size, shape and general appearance of the resulting daughter chromosomes. Thus while each of the spermatids formed as a result of this division receive only five chomosomes, the latter are bivalent and equivalent to ten of the somatic or spermatogonial chromosomes. There is some slight evidence that the secondary spermatocytes may occasionally divide with these chromosomes in their original condition of univalence.

5. Ten of the twelve chromosomes which passed to the other pole of the spindle in the primary spermatocyte behave in precisely the same way as described in the last paragraph. The two accessory chromosomes come to the equator of the spindle in the secondary spermatocyte with the five bivalents thus making in all seven. Each accessory now divides so that the resulting spermatids each receive seven chromosomes; that is, five bivalent plus two accessory, or the equivalent of twelve univalent chromosomes.

6. In reality, then, of the total number of spermatids, half have in all probability received ten, and half, twelve (10 plus 2) univalent chromosomes. Inasmuch as the spermatids transform directly into spermatozoa, there must be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes.

7. It is a significant fact that approximately half the resting spermatids when strongly decolorized after iron-hæmatoxylin staining, show two chromatin nucleoli and half do not. It seems probable that these nucleoli may correspond to the accessory chromosomes and are to be identified with the two

nucleoli of the primary spermatocyte and the two eccentric chromosomes seen in the spermatogonia.

8. It is probable that in man and certain other vertebrates, as in the insects, myriapods and arachnids, the accessory chromosomes are in some way associated with the determination of sex.

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EXPLANATION OF PLATE I.

All of the drawings were made with the aid of a camera lucida; their magnification is approximately 1,550 diameters. While the chromosomes are represented as accurately as possible, no attempt has been made to show details of the achromatic structures beyond general appearances and relations. In several cases where the chromosomes were not in the same focal plane they have been drawn carefully in the most favorable plane and then corrected as much as possible from second more accurate drawings of such individual chromosomes as were not in focus in the first drawing.

FIG. 1. Late prophase of spermatogonial division showing twenty-two chromosomes. The two chromosomes lying to one side of the main zone of chromosomes are presumably the two accessories.

FIG. 2. Nucleus of primary spermatocyte showing spireme stage with two chromatin nucleoli.

FIG. 3. Nucleus of primary spermatocyte showing the contraction-phase of the nuclear contents, also two persisting nucleoli.

FIG. 4. Late prophase of division in a primary spermatocyte showing twelve chromosomes. The two lying to one side of the main group are the accessories.

FIG. 5. Late prophase of division in a primary spermatocyte showing twelve chromosomes. Here the two accessories are not readily identified.

FIGS. 6, 7, 8, 9. Metaphases of divisions in primary spermatocytes showing the two accessories in characteristic position passing early to the poles. Fig. 7 shows also two precociously diverging daughter chromosomes.

FIG. 10. One end of a late anaphase of division in a primary spermatocyte showing twelve chromosomes (10 plus 2 accessory).

FIG. 11. Probably one end of a late anaphase of division in a primary spermatocyte, showing ten chromosomes, the accessory chromosomes having gone to the opposite pole; possibly a prophase of division in a secondary spermatocyte in which the ten chromosomes have remained univalent.

FIG. 12. Two contiguous secondary spermatocytes of which one shows five bivalent chromosomes in late prophase, the other more than five chromosomes (probably five bivalent plus two accessory) in metaphase. These two cells are evidently the product of a division of a primary spermatocyte in which ten chromosomes passed to one pole and ten plus the two accessories to the other.

FIG. 13. Two contiguous secondary spermatocytes. One, having just divided, shows five chromosomes at one pole; the chromosomes at the other pole are so massed as to preclude counting although there should be five. The other secondary spermatocyte shows seven chromosomes in late prophase.

FIG. 14. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes, showing seven chromosomes in all.

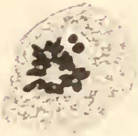
FIG. 15. Anaphase of division in a secondary spermatocyte showing still at the equator a lagging chromatic mass which is probably the two accessory chromosomes although it could not be positively identified as such.

FIG. 16. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes. Each of the latter divides as an independent chromosome at this time.

FIG. 17. One end of a late anaphase of a division in a secondary spermatocyte which had not received the accessory chromosomes.

FIG. 18. Two contiguous spermatids, one without chromatin nucleoli, the other with two. The spermatids in general are about equally divided into these two classes.

FIG. 19. Nucleus of a primary spermatocyte showing two chromatin nucleoli.



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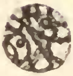

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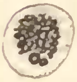

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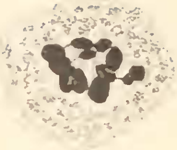

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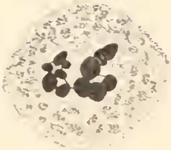

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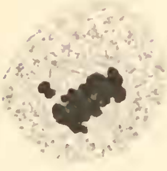

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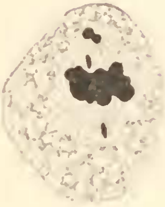

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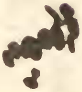

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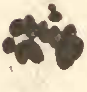

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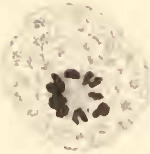

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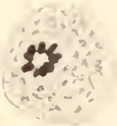

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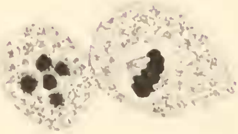

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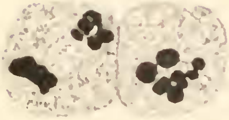

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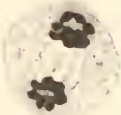

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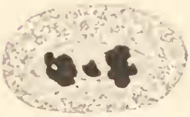

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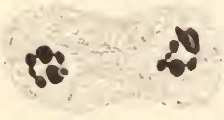

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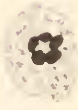

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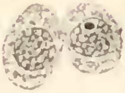

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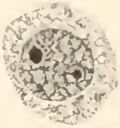

19

MICHAEL F. GUYER.

ON THE RIGHTING MOVEMENTS OF THE STARFISH.[1]

A. R. MOORE.

In an article entitled "The Behavior of the Starfish, *Asterias Forreri* de Lariol,"[2] Jennings discusses the movements of that animal in righting itself. He assumes that the starfish makes its movements in order to adapt itself to its environment and that therefore these movements are purposeful. From this assumption he concludes that "when the starfish is turned over on its dorsal surface locomotion is impossible, the finding and capture of food must stop; the delicate gills are pressed against the bottom, injuring them and impeding respiration; and displacements of the internal organs must occur that may be harmful to their proper functioning. We find, as might be anticipated, that there is a regulation of these bad effects by movement; the starfish turns again on its ventral surface."

A much simpler explanation of the righting movements has been given by Loeb.[3] He points out that the tube feet are positively stereotropic. Therefore the arms twist and turn until all of the tube feet are in a position to be in contact with a surface.

I have made observations on about thirty specimens of *Asterina miniata* and a like number of *Asterias ochracea*, with a view to determining the nature of the righting movements.

Let us look first at the causes, which Jennings has given us, for the starfish righting itself.

In regard to the first, viz., that locomotion is one of the ends which a starfish has in view in righting itself, I have found that very frequently the starfish crawls up the side of the aquarium and, upon reaching the surface of the water, thrusts out three or four arms dorsal side downward, their tube feet clinging to the surface film of the water. In such a position they remained sometimes for more than an hour, although further locomotion was impossible, and no attempt at righting was made. In fact, the starfish often retained such a slight attachment to the wall of the aquarium that the surface film of the water could no longer support the weight of the animal, with the consequence that the latter fell to the bottom of the tank. In such cases the tube feet cling to the surface film of the water because the film acts as a solid surface; it cannot, however, bear the animal's weight. Romanes[1] speaks of these movements as follows: "On reaching the surface, the animal does not wish (!) to leave its native element . . . and neither does it wish (!) again to descend into the levels from which it has just ascended. It therefore begins to feel about for rocks or sea weeds at the surface, by crawling along the side of the tank and every now and then throwing back its uppermost ray or rays along the surface of the water to feel for any solid support that may be within reach." Romanes evidently was not familiar with surface tension. Had he known that the surface film of a liquid acts like a solid surface he would have been prevented from attributing intelligence to the starfish.

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[1] From the Herzstein Research Laboratory, New Monterey, Cal.

[2] Jennings, *University of California Publications*, Zoology, Vol. 4, pp. 53–185.

[3] Loeb, "Comparative Physiology of the Brain," Chapter 3.

In order to see whether pressure on the gills might, as Jennings states, cause the starfish to turn over, I supported a glass plate in the aquarium, at a height just sufficient to press lightly on the dorsal side of a starfish moving over the floor of the tank. This was placed in the path of an approaching starfish. The latter did not change its direction when the plate was touched, but pursued its course, although the gills were pressed down. Furthermore, if a starfish is allowed to attach itself to a glass plate and is then suspended dorsal side downward so that it touches the bottom, its movements continue normally, although it could easily right itself if that were necessary. Clearly, then, pressure on the gills is not one of the factors which causes a starfish to right itself.

The displacements of the internal organs which, we are assured, "must occur" when the dorsal side is down, can only be due to gravity. I have frequently observed large numbers of starfish clinging, dorsal side downward, to overhanging ledges, feeding on barnacles and mollusks. Surely, "the displacements of the internal organs which *must* occur" when the dorsal side is downward, do not interfere in the least with the ingestion and digestion of food. Such "displacements" can, therefore, hardly be considered seriously as causes for the righting movements taking place.

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[1] Romanes, "Jellyfish, Starfish and Sea Urchins," p. 268.

We are forced to conclude, from the observations described, that, as Loeb has stated, the starfish ceases its efforts to right itself the moment all the tube feet can be brought into contact with a solid surface. Gravity plays no part in the righting movements.

The idea has been advanced by Loeb[1] that the mechanism of the righting movements is the result of coördinating and inhibiting impulses, which are transmitted to the various arms by the ventral nerve ring.

Six distinct methods of the righting reaction have been described by Jennings, but he has made no analysis of them on the basis of inhibiting and coördinating impulses. My observations agree with Loeb's assumption and give a rather simpler explanation of the behavior of the starfish.

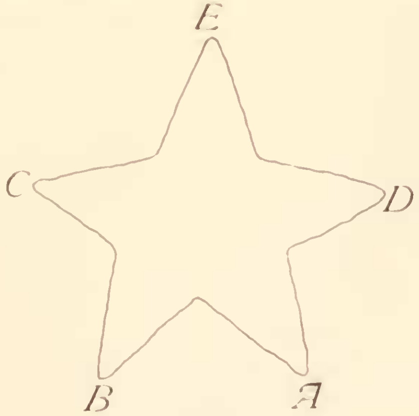

FIG. 1.

As a rule, when a starfish is placed upon its dorsal side, the arm whose tube feet first touch bottom determines the course of the righting. This arm begins at its distal end to twist the dorsal side upward, and as rapidly as the twisting is accomplished the tube feet secure a hold on the bottom. Next, if not simultaneously, the arm adjacent to the ventral side of the arm which is obtaining a hold, twists, so that the ventral surfaces of the two arms face each other, and secures itself in the same manner as the first. If *A* and *B* have in this way attached themselves to the bottom, inhibiting impulses are sent to *C* and *D*. The latter release themselves if they have already seized the bottom, rise ventrally, dragging *E* which either remains passive or bends dorsally, even catching the bottom with its tube feet in some cases. The righting is completed by *C*, *D* and *E* passing over *A* and *B* and attaching.

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[1] Loeb, "Comparative Physiology of the Brain," Chapter 3.

This simple and useful method of righting may be modified by (1) inequalities in the length of the arms, (2) injuries to certain of the arms, (3) any initial twist an arm may have due to its position before the animal was laid upon its dorsal side.

As to (1) short arms are more sluggish than ones of normal length, (2) injury to an arm inhibits the active twisting and seizing of the surface with the tube feet of this arm, (3) if an arm is partially twisted its tube feet reach the bottom more quickly than they otherwise would. As a result we have the following modifications of the normal method of righting.

1. If four arms are injured, their activity is inhibited and the righting is accomplished by the one uninjured arm. It may force an adjacent arm to coördinate weakly.

2. If *A* and *C* (Fig. 1) twist so as to face each other with their ventral surfaces, *B* receives two impulses, from opposite directions, to coördinate, and therefore does not twist either way but bends under dorsally, allowing *A* and *C* to accomplish what *A* and *B* did in the normal case. The same result may be brought about by injuring *B*, *D* and *E*.

3. Sometimes inhibitions are weak and *A*, *B*, *C* and *D* may all remain attached, *C* and *B* facing ventrally toward *A* and *D*. *E* alone is inhibited and the righting is accomplished by *A* and *B* walking backward under *C* and *D*.

I found, as Jennings noted, that in a few cases a starfish persistently refused to use a certain arm for initiating the righting movements. In most cases this was clearly due to an injury or malformation of the inactive arm. According to the author cited, such a starfish could be "taught" to use the idle arm by giving the animal a large number of "lessons" (180 in one case) in which arms ordinarily active were prevented from taking hold by "stimulating their tube feet with a glass rod" whenever they attempted to attach themselves.

I was able to compel starfish of this sort to use the idle arm by injuring the active ones in the following ways: (1) Irritating the ventral groove of the arm by rubbing it with a glass rod, (2) treating the tips of the arm with a few drops of $n/10$ acid. Two or three applications a few minutes apart usually sufficed to render the arm inactive. In this way I was able to "teach" the starfish in one "lesson," spontaneously to use an arm previously inactive. The length of time the lesson was "remembered" depended upon the degree of the injury. It seems evident from this that Jennings' "lessons" consisted merely in inhibitions due to the injury caused by his irritating the tube feet of the active arms. But an inhibition caused by a single or persistent stimulation is not identical with the phenomena of memory manifested in the process of teaching.

Summary.

1. The righting movements of a starfish which has been placed on its dorsal side are due only to the positive stereotropism of the tube feet.

2. An injury to an arm inhibits its being used for the initiation of righting movements.

3. A starfish cannot be taught to use an arm which is ordinarily passive, but by injuring the other four arms these can be prevented from initiating righting movements and the fifth arm then initiates these movements.

I wish to express my sincere thanks to Professor Loeb for his helpful suggestions and criticism.

A SIMPLE COOLER FOR USE WITH THE MICROTOME.

CASWELL GRAVE AND OTTO C. GLASER.

The microtomist's ability to prepare thin paraffin sections, depends, among other things, on the hardness of his imbedding medium, and this, in turn, on the temperature of the laboratory. Usually this circumstance offers no insurmountable difficulties, but there are times and places when this is not true. To meet such conditions several devices have been suggested and used by various investigators, but we know of none so simple, or as little likely to make difficulties, as the one about to be described.

The apparatus, which is shown, set up for action, in Fig. 1, is essentially a hollow truncated pyramid, open at both ends, and suspended in an inverted position from a standard, so adjusted that the lower end of the shoot is at a convenient distance above the knife. At the upper end of the inverted pyramid, and surrounded by it, is a tray whose dimensions are less than those of the base of the shoot. This tray is filled with crushed ice, and from one corner of it a drain leads the water to the escape from the lower end of the air-channel. At that point a rubber tube connects the pipe with a suitable receptacle.

The cooler is easily set up, interferes in nowise with the operator, and is thoroughly effective. When the air of the room strikes the melting ice in the tray, it is chilled and immediately falls between the tray and the walls of the pyramid. In this way a constant stream of cold air pours from the lower end of the shoot, and as this may be placed directly above the paraffin-block and knife-edge, both of these are cooled, and make it possible to cut sections very much thinner than the unmodified temperature of the room would allow.

The extent to which it is desirable to cool the paraffin and knife varies with each specific case, but the cooler is adjustable in at least two ways. In the first place the distance of the block from the end of the shoot can be changed within comparatively wide limits; in the second place the temperature of the air delivered may be further lowered by the addition of NaCl to the ice. Other salts can be used should a greater depression of the temperature be necessary.

The following table is the record of a test made at a room temperature of 87.8°F. The material in this particular case could not be imbedded in paraffin with high melting-point and satisfactory sections, even as thick as 12 micra, could not be cut. With the aid of the cooler however, a perfect series, 3 micra in thickness, was easily prepared from the same block of 45-48° paraffin.

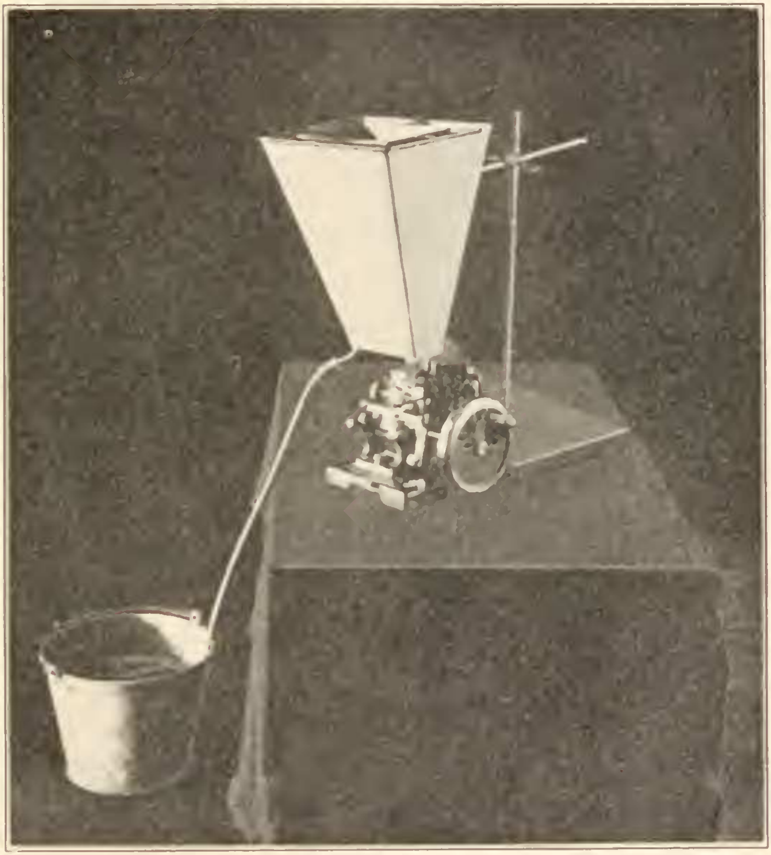

FIG. 1. Photograph of Cooler, by Miss Francis J. Dunbar. Measurements of pyramid: base, 12.5 in. x 8.7 in.; truncated apex, 6.1 in. x 2.1 in. Measurements of Ice-tray: 8.8 in. x 3.3 in. Cost, $3.50 without the standard.

TEST OF COOLER.

Room temperature ....31°C....87.8° F

Contents of Tray.	Distance below Mouth of Shoot.		
Crushed ice	6 cm.	24.5	76.1
Crushed ice + NaCl	6 "	23	73.4
Crushed ice	3 "	18	64.4
Crushed ice + NaCl	3 "	17	62.6

Several coolers, varying somewhat in size, but all modeled after our original one at Johns Hopkins University, are now in use in different laboratories. The measurements given in connection with Fig. 1, are those of the cooler at the University of Michigan. This particular one does not have the advantage of a removable ice-pan. In general, size is of little consequence unless it involves too great a reduction in the capacity of the ice-tray, or is conducive to too much absorption of heat by the sides of the pyramid. This latter difficulty is easily overcome by lining the shoot with asbestos paper.

JOHNS HOPKINS UNIVERSITY,
UNIVERSITY OF MICHIGAN.
July 15, 1910.

THE QUESTION OF REVERSAL OF ASYMMETRY IN THE REGENERATING CHELÆ OF CRUSTACEA.

CHARLES R. STOCKARD.

During the summer of 1909 while at the Tortugas Laboratory of the Carnegie Institution I undertook a further analysis of the reversal phenomenon in regenerating specimens of the genera *Alpheus* and *Synalpheus*. These small crustacea commonly called snapping shrimp, on account of their habit of snapping the large chelæ with such force as to produce a surprisingly loud noise, are abundantly found in the "logger-head" sponge and in the holes of disintegrating coral rock on the Tortugas reefs. There are a number of species five of which, *Alpheus formosus* and *armillatus* and *Synalpheus minus* and two other unidentified species of *Synalpheus*, were employed in these experiments.

The several species differ in size and body color but are essentially similar in general structure. The first pair of appendages is decidedly asymmetrical in both sexes. One member of the pair, either the right or the left, is extremely large, in some cases being more than half the size of the body itself. The general type of this chela in the five species follows more or less closely the description given by Wilson,[1] for the great chela of *Alpheus heterochelis*. It is greatly rounded or swollen with transverse grooves on either side of the propodus, varying in depth with the species, and presents characteristic color patterns being tipped with a lively rose color in *Synalpheus minus* while in the other species it is bluish, dark or brown. On the concave side of the dactylus is a swollen knob forming the "hammer" which fits into a corresponding socket on the outer side of the propodus claw. By extending the dactylus and then suddenly snapping the claws together the "hammer" is forced into the socket with the surprisingly loud sound.

Wilson's description further applies in that the large chela has essentially the same structure in both sexes, while the small chela shows characteristic sexual differences, we shall be mainly concerned, however, with the fact that the small chela is always typically different from the large chela in shape as well as in size.

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[1] Wilson, E. B., "Notes on the Reversal of Asymmetry in the Regeneration of the Chelæ in *Alpheus heterochelis*." BIOL. BULL., IV., pp. 197–210, 1903.

Przibram[1] discovered that in several species of *Alpheus* after the removal of the large chela a chela of the small type regenerated from its base while the small chela of the opposite side metamorphosed or developed into a great chela of typical form at the following moult. In other words, the asymmetry was reversed. Further, when both first chelæ are removed they regenerate in their original conditions, no reversal following.

Zeleny[2] found an exactly similar phenomenon to occur after removal of the functional operculum in the worm, *Hydroides*. In this case the rudimentary operculum of the opposite side develops into a functional operculum while a rudimentary organ regenerated from the base of the former functional one. The principle involved in this reversal phenomenon is doubtless the same as that in the crustacea.

Przibram[3] later found a similar reversal to occur in other species of crustacea, while in others the removal of either chela is followed by the regeneration of one of the simpler or smaller type without a regulatory change taking place in the uninjured chela of the other side. In still other cases, as for example the lobster, *Homorus*, a chela similar to the one removed invariably regenerates whether the original chela was a large crusher claw or the slender nipping claw.

The crustacea thus present a series from those forms which regenerate appendages of the type of the ones removed, others which regenerate appendages of the simpler type without a compensatory change taking place in the uninjured chela, and finally such forms as *Alpheus* in which the simpler type of chela is regenerated after the removal of the more specialized chela while the uninjured small chela develops into the more modified type, and thus by a sort of compensatory regulation the animal's asymmetrical condition is quickly reëstablished.

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[1] Przibram, H., "Experimentelle Studien uber Regeneration," *Arch. fur Entw.-Mech.*, XI., 1901.

[2] Zeleny, C., "A Case of Compensatory Regeneration in the Regeneration of *Hydroides dianthus*," *Arch. fur. Entw.-Mech.*, XIII., 4, 1902.

[3] Przibram, H., "Experimentelle Studien über Regeneration, II.," *Arch. für Entw.-Mech.*, XIII., 1901–1902; "Equilibrium of Animal Form," *Jour. Exp. Zoöl.*, V., p. 259, 1907–1908.

Wilson repeated Przibram's experiments on *Alpheus heterochelis* with similar results, but carried the experiments further, hoping to analyze the factors concerned in the reversal process. After removing the great chela the nerve trunk leading to the small chela of the opposite side was clipped in order to test whether there was a nervous control determining the growth of the small chela into a large one. After such an operation the small chela was generally thrown off and only two specimens are said to be beyond question, yet "one of these did not moult quite normally and the other not at all." The evidence, then, does not warrant conclusions as to the cause of reversal of asymmetry in the chelæ. Wilson finally believes that the initial factor that sets in motion the complex process of differentiation of which either side is capable, is primarily only a difference in the amount of material on the two sides. "Removal of the large chela obviously reversed the asymmetry in respect to the amount of material and must, temporarily, at least, lead to a functional nervous difference." Such a suggestion may easily be submitted to experimental test, for example, after removal of the large chela from one side of the body if several posterior appendages be removed from the other side the greater amount of material may still remain on the original large chela side. Fig. 1, *A*, illustrates the operation. Under these conditions will a large chela regenerate from the stump of the old one, instead of arising by a growth of the small first chela of the opposite side?

Again, the proposition may be tested by removing both the great and small chelæ of the first pair and in addition amputating several legs on the side of the large chela; the operation is illustrated by Fig. 1, *E*. The greater amount of material is now on the original small chela side; will this extra amount cause a great chela to regenerate from the small stump instead of from the stump of the great chela which is on the side with less material?

Lastly, when only a portion of the great chela is amputated does it regenerate in the original condition or become a small chela, while a large chela appears on the opposite side through a metamorphosis of the small first chela?

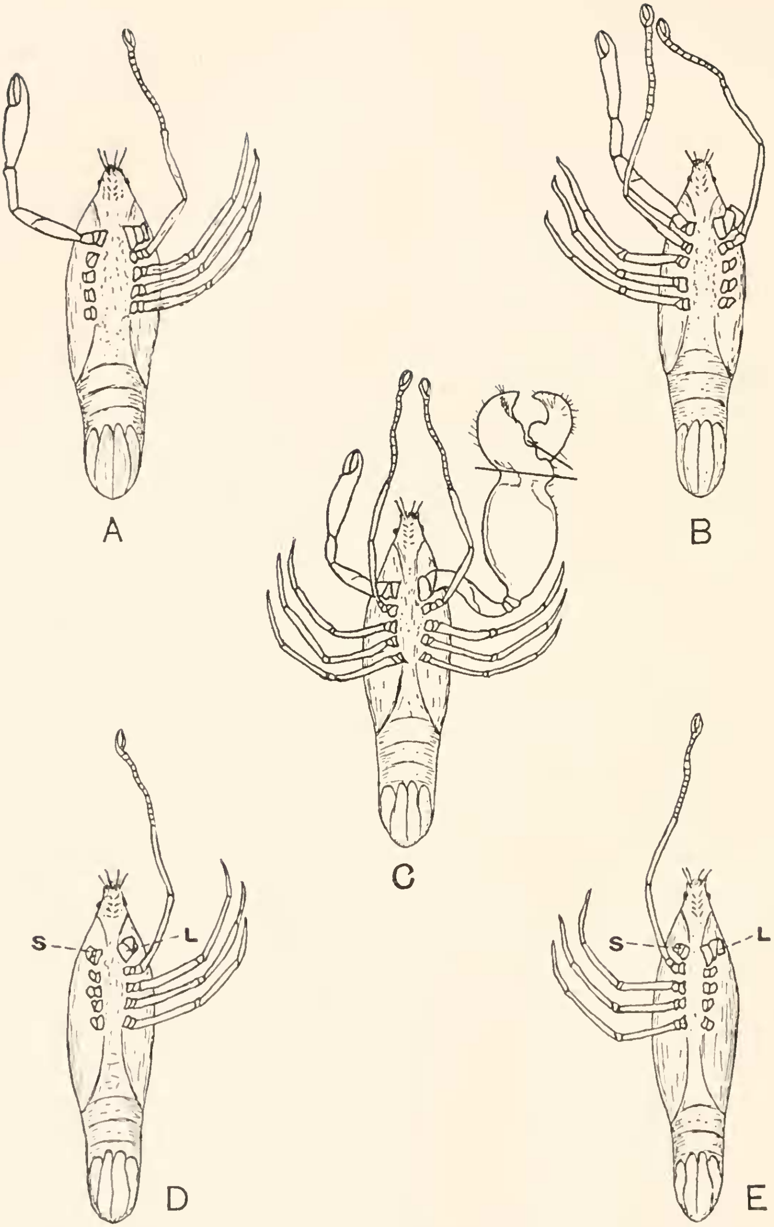

FIG. 1. Diagrams illustrating the manner of operation in the several experiments. *A*, removal of the great chela and the posterior appendages of the opposite side, causing the larger amount of material to still remain on the great chela side; *B*, the opposite operation as a control; *C*, the heavy lines show the places at which portions of the great chela may be cut away without reversal; *D* and *E*, removal of both first chelae and posterior appendages from either the small, *D*, or great, *E*, chela side, to determine the influence of the lateral amount of material on the regeneration of the first chelæ; *L*, large chela stump; *S*, small chela stump.

Aiming towards an answer for these questions a number of experiments were performed the results of which may now be considered.

Fifty healthy specimens representing the five different species were selected, and tested as to their tendency to reverse the asymmetry of the first pair of chelæ during the regeneration following the removal of the great chela. Without exception all of the specimens responded as Przibram had found, a small chela regenerated from the stump of the original great one and the small chela of the opposite side metamorphosed into a great chela. Forty of the specimens favorably survived the experiments.

FIRST SERIES.

Nineteen individuals had the great chela removed and in addition a number of posterior appendages were amputated from the opposite or small chela side, so as to allow the greater mass of material to remain on the original great chela side. The operation is illustrated by Fig. 1, *A*, and the opposite or control operation by Fig. 1, *B*, or specimens 10, 12 and 31 in the table. Referring to the table the results of such an experiment may be ascertained.

The left side of the table gives the date of the operation, the number of the specimen, and the appendages removed are indicated by *x*, *G* and *S* in the first appendage column indicate the great and small chela. The right side of the table gives the time of moulting and the manner of regeneration, the *r* signifies a new or regenerated leg, *G* and *S* in the first appendage column again indicate the great and small chela. The horizontal lines of the table are so arranged that the appendages on the right side of an individual are given immediately above those on the left of the same animal, *e. g.*, specimen 1 had the great chela and the second leg removed from the right side and the fourth leg from the left side in the first instance. Each specimen, as the table shows, was operated upon a second time during the experiments.

Of the nineteen cases having the great chela removed from one side and other appendages than the first chela from the opposite side the small chela, even though it was on the side of less material, retained the power to grow into a large chela of typical form in seventeen cases. One of these cases, specimen 23, is remarkable, since at the first operation the small chela which was on the left side and the other four left appendages were all removed and regenerated at the next moult. After this moult the large chela of the right side was amputated along with the four posterior legs of the left side, thus leaving only the regenerated small chela on the *left side* while the four posterior legs remained on the right side. Nevertheless, the left small chela grew into a great chela and the four more posterior left appendages regenerated for the second time. The case seems an extreme test of the power of one side to regenerate all of its posterior legs for a second time and in addition to change the first chela from the small to the large size and type. Specimen 25 further indicates this remarkable power of the side of the individual with less material to replace all lost parts and at the same time increase the size and type of its first chela.

The remaining two of the nineteen cases, specimens 9 and 18, present the first chelæ equal in size after the moult following the second operation. Specimen 9 had in the first case the great chela removed from the right side and the third, fourth and fifth appendages from the left side. The greater amount of material was, therefore, still on the right side, yet the small first chela of the left side became larger after the moult. The second operation removed the great left chela and the second, third, fourth and fifth legs of the right side. After the moult all of the amputated legs were regenerated but the regenerated left first was small, and the right first appendage had not increased in size. Thus the first pair were symmetrical in respect to size yet the right first or old chela had slightly approached the large chela type. The specimen 18 responded in a closely similar fashion.

The type of the chela is equally, if not more, important than the size since Przibram found the chelae to be of almost equal size in some cases but of reversed type, and the great type invariably increases in size at the following moult.

The experimental evidence in the first case, then, does not support the idea that the side with most appendage material has most power to produce a great chela of typical size and form. The tendency is to produce a chela of the great size and type from the uninjured first chela, even though this be the original small chela and is located on the body side which has suffered the loss of all other walking appendages. There seems to be no evidence from these experiments to suggest a bilateral distribution of growth energy accompanying distribution of appendage material.

Second Series.

The question of a bilateral distribution of growth energy related to, or accompanying, the amount of appendage material on a given side was further tested in the following manner. Fifteen specimens were operated upon so as to remove both chelæ of the first pair. It was known that when only these two chelæ were removed that they regenerated in their original condition, a large chela from the base of the original large chela and a small chela from the opposite side. If now in addition to the removal of the first pair of appendages a number of more posterior appendages also be removed from the large chela side, this side will have less appendage material remaining and is, therefore, called upon to regenerate a greater amount of material to replace the posterior legs. Will this side of the body at the same time be more capable of producing a first chela of larger size and specialized type than the opposite side which is called upon to replace only the first chela? Such an operation is illustrated by Fig. 1, *E*, and Fig. 1, *D*, forms a control experiment in which the additional appendages are removed from the small chela side.

The usual idea of regulation would require the side with only the first leg removed to regenerate a large chela while the other side replaced the several posterior legs and produced a small first chela. It is found on examining the table that after an operation to remove both first chelae and one or more posterior legs on *either* the great or small chela side that six of fifteen specimens regenerated the chelæ of the first pair equal in size, not one individual reversed the type of the first chelæ, and eight regenerated the first chelæ in their original condition as though no additional appendages had been removed. One specimen died before the experiment was completed.

Considering the six specimens that regenerated the chelæ of the first pair equal in size it is important to find that four of these cases, specimens 16, 20, 21 and 39, had the larger number of posterior appendages removed from the side of the original small chela and not from that of the large chela, so that the greater amount of material remained on the large chela side. Such operations were intended as a control for the results following the removal of posterior appendages from the large chela side. Although in these four specimens there was more material on the original large chela side and this side was called upon to regenerate fewer appendages it failed to produce a great chela from the stump of the original one.

In specimen 16 the first chelæ remained equal in size and were both of the small chela type after a second moult. The left chela was then removed and after the next moult the right developed into a great chela and the left again regenerated small. Both first chelæ in specimens 20 and 21 were also of the small chela type, while in specimen 39 the small chela failed to regenerate at the first moult after the operation though a chela of the small type regenerated from the base of the great chela and remained small while the right small chela regenerated at the next moult.

Specimens 31 and 32 had both first chelæ and a number of appendages, four and three, on the great chela side removed. After the moult following the operation the first chelæ were equal in size. Yet again specimens 27, 35 and 38 were operated upon in an identical fashion and after the moult they were able to produce a great chela from the original great chela stump even though this side was called upon to regenerate three other appendages.

Of the fifteen cases tried, therefore, eight regenerate their first chelæ in the original condition of asymmetry while six regenerate the chelæ of the first pair equal in size and usually similar in type whether additional appendages are amputated from the great chela side or from the small chela side. Such a fact would seem to indicate that the amount of appendage material present on either side is an unimportant factor in determining the type of the first chela on a given side, and it seems to show further that there is no clearly evident bilateral distribution of growth energy in these regenerating specimens.

TABLE I.

The Effect of Regeneration on the Asymmetrical Condition of the First Pair of Chelæ in Five Species of *Synalpheus* and *Alpheus*.*

Date of Operation.	Specimen Number.		Appendages.					Date of Moult.	Appendages Regenerated					Remarks.
			1	2	3	4	5		1	2	3	4	5	
May 24	1	R	Gx	x				June 4	Sr	r				
		L				x			G			r		
June 6	1	R		x	x	x		June 16	G		r	r	r	The small chela increases in size and changes its form to type of great chela.
		L	Gx						Sr					
May 24	2	R						June 1	G					
		L	Sx	x		x			Sr	r		r		
June 6	2	R	Gx	x				June 15	Sr	r				The third and fifth are only short buds, not fully regenerated.
		L		x	x		x		Gr	r	r		r	
May 24	3	R			x	x		May 30	G		r	r		
		L	Gx						Sr					
June 6	3	R	Gx	–				June 15	Gr	–				Only the dactyl removed and regenerated.
		L							S					
May 24	4	R	Gx					May 29	Sr					
		L							G					
June 6	4	R			x	x	x	June 13	G		r	r	r	Also moulted one day after the operation but had not regenerated.
		L	Gx						Sr					
May 24	5	R						June 4	G					
		L	Gx						Sr					
June 6	5	R	Gx	x				June 13	Sr	r				Failure of third and fourth legs to regenerate might account for first chela becoming large.
		L		x	x	x	x		G	r	o	o	r	
May 24	6	R	Sx	x	x	x		June 1	Sr	r	r	r		
		L	Gx						Gr					
June 6	6	R		x	x	x	x	June 12	G	r	r	r	r	
		L	Gx						Sr					
May 24	7	R						June 3	G					Moulted few hours after operation on May 24 and right first chela began to increase in size.
		L	Gx		x				Sr		r			
June 6	7	R	Gx					June 13	Sr					
		L			x	x	x		G		r	r	r	
May 24	8	R	Gx					May 30	Sr					
		L							G					
June 7	8	R				x	x	June 14	G			r	r	Also moulted two days after operation but no regeneration.
		L	Gx						Sr					
May 24	9	R	Gx					May 29	Sr					Moult followed so soon after operation that left first chela had not attained full size, but did so after a few days.
		L			x	x	x		G		r	r	r	
June 7	9	R		x	x	x	x	June 14	S	r	r	r	r	Also moulted 2 days after operation. Right first somewhat similar to great chela type but small.
		L	Gx						Sr					
May 24	10	R						May 30	G					
		L	Gx	x		x	x		Sr	r		r	r	

TABLE I.—*Continued.*

Date of Operation	Specimen Number.		Appendages.					Date of Moult.	Appendages Regenerated.					Remarks.
			1	2	3	4	5		1	2	3	4	5	
June 7	10	R	Gx	x				June 14	Sr	r				Operated immediately after a moult on June 7.
		L				x	x		G			r	r	
May 24	11	R			x	x		May 30	G		r	r		
		L	Gx						Sr					
June 7	11	R	Gx	x				June 13	Sr	r				
		L			x	x	x		G		r	r	r	
May 24	12	R	Gx					June 2	Sr					
		L		x	x	x			G	r	r	r		
June 7	12	R						June 15	G					
		L	Gx		x	x	x		Sr		r	r	r	
May 24	13	R	Gx				x	May 29	Sr				r	
		L					x		G				r	
June 7	13	R												Died after operation.
		L	Gx											
May 24	14	R	Gx				x	June 1	Gr				o	Only the dactylus and index cut from great chela, no reversal.
		L												
June 7	14	R	Gx	–				June 14	Gr					Second operation and result the same as first.
		L												
May 24	15	R	Gx					June 3	Gr					
		L	Sx						Sr					
June 7	15	R	Gx	x				June 15	Sr	r				
		L			x		x		G		r		r	
May 24	16	R	Gx				x	May 31	Sr				r	After a second moult on June 7 the first chelae were still of equal size.
		L	Sx			x	x		Sr			r	r	
June 7	16	R						June 13	G					The left first chela was removed and right became large and left regenerated small.
		L	Ex						Sr					
May 25	18	R			x			June 1	G		r			
		L	Gx						Sr					
June 7	18	R	Gx					June 14	Sr					Pincer of left first seems slightly nearer the great chela type.
		L			x	x	x		S		r	r	r	
May 25	19	R	Gx					June 3	Sr					
		L							G					
June 8	19	R	Sx		x			June 15	Sr		r			
		L	Gx			x	x		Gr			r	r	
May 25	20	R			x	x	x	June 6	G		r	r	r	
		L	Gx						Sr					
June 8	20	R	Gx					June 19	Sr					Both first chelae of the small type and equal in size.
		L	Sx			x	x		Sr			r	r	
May 25	21	R		x		x	x	May 30	G	r		r	r	
		L	Gx						Sr					
June 8	21	R	Gx	x				June 13	Sr	r				Both first chelae of equal size and small type.
		L	Sx	x		x			Sr	r		r		
May 25	23	R		x		x		June 4	G	r		r		
		L	Sx	x	x	x	x		Sr	r	r	r	r	
June 7	23	R	Gx					June 15	Sr					Second regeneration of all posterior left legs, yet left first became large chela.
		L		x	x	x	x		G	r	r	r	r	
May 25	25	R			x	x	x	June 1	G		r	r	r	
		L	Gx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages.					Date of Moult.	Appendages Regenerated.					Remarks.
			1	2	3	4	5		1	2	3	4	5	
June 10	25	R	Gx		x	x	x	June 17	Gr		r	r	r	Again second regeneration on one side yet first chela of this side becomes large.
		L	Sx			x			Sr			r		
May 25	26	R						June 6	G					
		L	Gx		x				Sr		r			
June 10	26	R	Gx											Did not moult the second time.
		L				x	x							
May 25	27	R		x	x	x		June 4	G	r	r	r		
		L	Gx			x			Sr			r		
June 10	27	R	Gx		x	x	x	June 17	Gr		r	r	r	
		L	Sx						Sr					
May 25	28	R	Gx				x	June 2	Sr				r	
		L							G					
June 8	28	R		x	x	x	x	June 14	G	r	r	r	r	Second pair of legs regenerated shorter than usual.
		L	Gx	x					Sr	r				
May 25	29	R	Gx					June 6	Sr					Moulted day before the operation.
		L							G					
June 8	29	R			x	x	x	June 16	G		r	r	r	
		L	Gx						Sr					
May 25	30	R					x	May 29	G-				r	Moult followed so soon after operation that little increase in right chela, no regeneration of left, after second moult June 7 condition complete
		L	Gx	x					o	r				
June 10	30	R	Gx		x	x	x							Died June 16 without moulting.
		L	Sx											
May 25	31	R						June 3	G					
		L	Gx	x	x		x		Sr	r	r		r	
June 10	31	R	Gx	x	x	x	x	June 18	Sr	r	r	r	r	First chelæ equal in size and of small type
		L	Sx						Sr					
May 25	32	R			x	x	x	May 31	G		r	r	r	
		L	Gx						Sr					
June 10	32	R	Gx		x	x	x	June 15	Sr		r	r	r	Both first chelæ smaller than normal but of equal size.
		L	Sx						Sr					
May 25	33	R		x	x	x		May 30	G	r	r	r		
		L	Gx						Sr					
June 10	33	R	Gx	x	x	x		June 16	Gr	r	o	o		Failure of 3 and 4 to regenerrate may account for first chela's growth.
		L	Sx						Sr					
May 25	35	R	Gx					May 29	Sr					
		L			x				G		r			
June 8	35	R	Sx					June 14	Sr					Left first chela great though regenerating three other legs on same side.
		L	Gx	x	x		x		Gr	r	r		r	
May 25	36	R	Gx					June 3	Sr					Also moulted 2 days after operation.
		L			x	x			G		r	r		
June 8	36	R			x	x		June 15	G		r	r		
		L	Gx						Sr					
May 25	37	R						May 30	G					Moulted again June 6.
		L	Gx			x			Sr			r		
June 8	37	R	Gx		x	x	x	June 15	Gr		r	r	r	
		L	Sx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages.					Date of Moult.	Appendages Regenerated.					Remarks.
			1	2	3	4	5		1	2	3	4	5	
May 25	38	R						May 30	G					
		L	Gx		x	x			Sr		r	r		
June 8	38	R	Gx	x	x	x		June 15	Gr	r	r	r		Also moulted June 9 day after operation.
		L	Sx						Sr					
May 25	39	R	Sx	x	x			June 6	o	r	r			
		L	Gx			x			Sr			r		
	39	R						June 14	Sr	r	r			First chelæ finally regenerated equal in size and remained so after next moult
		L							Sr			r		
May 25	40	R	Sx					May 30	Sr					Moulted again June 6.
		L	Gx			x	x		Gr			r	r	
June 8	40	R	Sx			x	x	June 15	Sr			r	r	
		L	Gx						Gr					

Each specimen was operated upon twice as indicated. R and L following the specimen number signifies right and left sides of the animal; G indicates the great and S the small first chela; x indicates the appendages removed and r the appendages regenerated.

Third Series.

Finally, an attempt was made to determine how large a portion of the great first chela might be removed without causing it to regenerate small; or to cause the small chela of the opposite side to grow into the great type. When a large portion of the chela was quickly clipped off with sharp scissors or a knife the remaining portion was soon thrown off at the breaking joint. The only successful operations consisted in the removal of the dactylus or most distal segment which forms part of the claw, and in the removal of the entire pincer or dactylus and distal end of the propodus, as is indicated by the lines drawn across the chela in Fig. 1, *C*. In the last case a stump-like appendage without a pincer remains.

Following either of these operations the great chela was fully reformed or renewed at the next moult, no reversal taking place.

A small portion of the great chela may then be regenerated in its original form. When the entire chela is removed the small chela of the opposite side invariably grows into a great chela and a small chela regenerates from the stump of the original great one. This reversal of asymmetry may be shifted back and forth for a number of times and occurs in a manner as decidedly pronounced after several operations as it does after the first.

CONCLUSIONS.

The power to reverse the asymmetry of the first chelæ when regenerating a great claw in *Alpheus* does not seem to be closely associated with a difference in the amount of material on the two sides of the body nor with a bilateral distribution of growth or regenerative energy.

Although in certain cases there seems to be a tendency to regenerate the chelæ of the first pair equal in size and similar in type, such a tendency is manifest under conditions so varied in respect to the bilateral distribution of appendage material and call upon the powers of regenerative energy that the present conclusion is warranted. The amount of material on a given side of the animal, or the amount of regeneration required of this side are negative factors in determining the ability of the side to produce a great chela instead of a small one.

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EXPERIMENTS ON COLOR-VISION OF THE HONEY BEE.

C. H. TURNER.

INTRODUCTION.

Whether insects can or cannot distinguish colors is a matter of much theoretical importance, for the correct interpretation of the relation of insects to flowers depends upon this answer. Most students of natural selection believed, at one time, that the forms and colors of flowers were adaptations to insect visitors. Lately there has been a reaction based on the general consensus of opinion, among morphological entomologists, concerning the poorness of insect vision. Kellogg[1] writes: "The fixed short focal distance, the incompleteness and lack of detail incident to a mosaic image, and the lack of accommodation (only partly provided for by the shifting of the peripheral pigment) to varying light intensity, which are admitted conditions of insect vision, make it seem difficult to account for the intricacy in pattern common to many flowers on a basis of adaptation to animal visitors of such poor seeing capacity as insects.

"Experimental evidence touching this criticism is singularly meager when one considers the importance of the subject. If insects can accurately distinguish colors, and at some distance, and can perceive the fine details of color-pattern at a very short distance, then the explanation of floral structure and pattern as adaptation to insect visitors has solid foundation for even the amazingly large and varied results which it attempts to explain; if not, it is hard to understand how the explanation is valid (at least in any such all-sufficient degree as commonly held), despite its logical character (in light of our knowledge of the nearly limitless capacity for modification of natural selection) and the abundant confirmatory evidence.

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[1] Kellogg, V. L., "American Insects," Henry Holt & Co., second edition, revised, 1908, pp. 580.

"Most of the experimental evidence so far offered is that included in Darwin's account ('On the Fertilization of Flowers by Insects'); in Lubbock's account of his experiments on honey-bees, familiar because of its presentation in his readable book, 'Ants, Bees and Wasps'; and in Plateau's account of his more recent but less familiarly known experiments with various insects including bees. Both Lubbock and Plateau are investigators ingenious in device, keen in deduction, and of unquestioned scientific honesty. Yet their conclusions are a direct contradiction. Lubbock believes that bees recognize colors at a considerable distance, that they 'prefer one color to another, and that blue is distinctly their favorite.' Plateau finds that neither the form nor the brilliant colors of flowers seem to have any important attractive rôle, 'as insects visit flowers whose colors and forms are masked by green leaves, as well as to continue to visit flowers which have been almost totally denuded of colored parts'; that insects show no preference or antipathy for different colors which flowers of different varieties of the same or of allied species may show; that flowers concealed by foliage are readily discovered and visited; that insects ordinarily pay no attention to flowers artificially made of colored paper or of cloth whether these artifacts are provided or not with honey, while, on the contrary, flowers artificially made of living green leaves and provided with honey are visited (from the attraction of the 'natural vegetable odor'). From these observations Plateau concludes that 'insects are guided with certainty to flowers with pollen or nectar *by a sense other than that of vision and which can only be that of smell*,' and finds particular proof of this in the facts, according to his observations, (1) that insects tend, without hesitation, towards flowers usually neglected by reason of the absence or poverty of nectar, from the moment that one supplies these flowers with artificial nectar, represented by honey; (2) that insects cease their visits when one cuts out the nectary without injuring the colored parts, and re-begin their visit if one replaces the destroyed nectary by honey; (3) that it suffices to attract numerous insects if one puts honey on or in normally anemophilous flowers, simply green or brown in color, which are normally practically invisible and almost never visited by insects; and (4) that the visiting of flowers artificially made of fresh green leaves and containing honey demonstrates plainly the rôle of the sense of smell.

"It must be said that, despite many just criticisms that may be made on the character of his experiments, Plateau has made necessary more experimentation for the relief of the general theory that floral adaptation of color is due to color preferences of insect visitors."

Forel[1] and von Buttel-Reepen[2] are opposed to Plateau's views, but Bethe[3] is in accord with Plateau.

To test his conclusions, Forel repeated, in the following manner, Plateau's dahlia experiment. (1) Paper dahlias were distributed among some dahlias from which a large number of bees were collecting honey. The bees paid no attention to these artifacts. (2) Honey was placed on these artifacts, and, by skillful manipulation, brought to the attention of one of the bees. Immediately that bee neglected the real dahlias for these artificial ones. (3) Gradually all of the bees neglected the dahlias for those artifacts with their inexhaustible supply of honey—inexhaustible because it was constantly replenished by Forel. (4) The artifacts were removed. After a lapse of several days, similar artifacts, but containing no honey, were scattered among those dahlias. Immediately the bees neglected the dahlias for the artifacts, which they searched for honey. Forel thinks this experiment shows that bees have space, form and color perception.

Von Buttel-Reepen bases his opposition to Plateau's views largely upon information furnished him by Herr Roth, leader of the Baden bee-keepers school, and a teacher named Staehelin.

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[1] Forel, Aug., "Die psychischen Faehigkeiten der Ameisen und einiger anderer Insekten," Muenchen, 1901. "Ants and Some of their Instincts," *Monist*, vol. 14, 1903-4.

[2] Buttel-Reepen, H. von, "Sind die Bienen Reflex-maschinen?, Experimental Beiträge zur Biologie der Honigbiene," *Biol. Centralbl.*, Bd. 20, 1900. "Are Bees Reflex Machines?" translated by Mary H. Geisler, Medina, O., 1907.

[3] Bethe, A., "Die Heimfähigkeit der Ameisen und Bienen zum Theil nach neuen Versuchen," *Biol. Centrlbl.*, Bd. 22, 1902.

Von Buttel-Reepen states: "We have seen above that the flight [of bees] becomes very unsafe in the dusk; therefore it is evident that gloomy weather influences considerably the ability to orient. 'One of my former neighbors,' Roth says in his communication, 'painted the gable of his house over the apiary with a sky-blue (luftblau) color. The same bees which always flew over the gable, on the next dark day, bumped against it with their heads, trying to fly through it.' A teacher, Staehelin, made the following observations: A weak after-swarm, mostly of young bees from a hive painted blue, dispersed among the masses of humming bees which were just taking their flight of orientation out of the other hives (which, as is usually the case in Germany, Switzerland, and Austria, were standing close together), and settled here and there in clumps. After a short time they flew back to the bee-house; but only a few found the right hive; the rest flew to other colonies, and to which? Only to those where a blue door invited them did they attempt an entrance, but nowhere else. Unfortunately they were so hostilely received that the ground in front of all of the blue hives was covered with bees."

Bethe had a swarm of bees lodged in a brown hive which rested on a table. He painted the outside of the hive blue and covered the table with green branches. Instead of the backgrond of trees, he substituted one of white and yellow flowered cloth. No change was produced in the home-coming of the bees. This Bethe considers conclusive proof that bees are not guided home by memory picture contributed by the eyes.

So far as my knowledge goes, M. Gaston Bonnier[1] is the only recent investigator who furnishes any experimental evidence that supports Bethe's view. He found that bees, the eyes of which had been rendered opaque with pigmented collodion, would pass direct to the hive from any distance less than three kilometers. This observation, which is not in harmony with Forel's experience,[2] supports Bethe's contention, but it has no direct bearing upon color vision.

The purpose of this paper is not to discuss the homing of the honey bee; but, by means of simple experiments, to throw some light upon the question "Can bees distinguish colors?"

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[1] Bonnier, M. Gaston, "Le sens de la direction chez les abeilles," *C. R. Acad. Sci.*, Paris, T. CXLVIII., 1909, pp. 1019–1022.

[2] Forel always found that bees, the eyes of which had been rendered opaque, could not find their way home.

Description of the Experiments.

The following experiments were performed in a large field just west of O'Fallon Park, St. Louis, Mo. The white sweet clover (*Melilotus alba* Lam.), with its long racemes of white papilionaceous flowers, was abundant in dense patches; but there were a few vacant places in the field. Foraging bees were visiting this white melilot in large numbers.

Series I. (July 12, 2 P.M.).

The discs used in this series of experiments were cut from colored cardboard, and each was six centimeters in diameter.

Experiment 1.—*I placed six discs of red cardboard on the top of rods that had been erected in the midst of a patch of white sweet clover. The rods were so adjusted that the top of each was about on a level with the tops of the weeds. Six similar discs were attached, at different heights, to the branches of the weeds. Honey was placed on all of these discs.*

More than an hour passed by and no response was made to these discs by the bees; but both flies and wasps visited them. The weeds were full of bees that were continuously flying to and fro in the immediate vicinity of these artifacts with their copious supply of honey. Were the odor of honey alone sufficient to attract bees reflexly, these bees should have been attracted early. After waiting half an hour, I decided to force the bees to attend to my artifacts.

Experiment 2. *A bee was captured in a wide-mouth bottle and the bottle, with the cork removed, inverted over one of the red discs of experiment 1, until the bee dropped upon the disc; the bottle was then removed. This was tried with six different bees.*

In each case the bee always ascended to the top of the bottle and attempted to escape. After several futile efforts it would drop, either by accident or from exhaustion, upon the disc. At that moment, I always removed the bottle. Immediately the bee would leave never to return. Some of the bees fell into the honey; but, even in that case, they did not return.

Experiment 3.—*A branch containing blossoms on which a bee was foraging was gently removed from the plant and so manipulated that the bee was less than two centimeters from the honey of one of the discs of experiment 1. This was tried with six bees.*

In no case did the bee pay any attention either to the honey or to my discs. The bee always left immediately and went to one of the blossoms of the melilotus.

Experiment 4.—*Whenever a bee alighted on a blossom near one of the discs of experiment 1, I gently moved the sprig until the bee was brought to within less than two centimeters of the honey. This was tried with a dozen bees.*

No response was made to the honey.

In a cluster of weeds about a yard from the one in which most of my discs were located, I had placed, at the beginning of this series of experiments, a red disc so copiously supplied with honey that it overflowed upon the weed. This disc was so situated that by simply raising my eyes I could see it. Although the melilotus was swarming with bees, that disc remained in that place for nearly two hours before receiving its first visit from a bee. At that time, however, a bee hovered at the edge of the disc and began to sip the honey. It then alighted on the edge of the disc and continued to sip the honey. Almost immediately another bee flew up to this one. They both circled about for a moment and then alighted on the disc; one on the edge and the other near the center of the upper surface. From this time on, all of my attention was focused upon this plant.

Experiment 5.—*Near this disc was a blossom which I had wet with honey. While the two bees mentioned in the above experiment were foraging on disc one, a bee alighted on this blossom. I gently moved the sprig until the bee was within about a centimeter of the two bees just mentioned.*

It left the blossom and, alighting on the disc, began to forage.

Experiment 6.—*While these bees were imbibing honey, I attached two other red discs, each supplied with honey, to other branches of the weed.* (For descriptive purposes, starting with the disc upon which the bees were feeding, we will designate them disc one, disc two, disc three.)

One by one, the three bees on disc one departed for the hive.

On leaving, each hovered a moment above the disc, circled around it, made two or more short circles about the weed, then, ascending, departed. In about five minutes, two of the bees had returned to the weed. They did not visit any of the blossoms; but, after circling in the vicinity of the disc for a moment, alighted on the disc and began to imbibe the honey. They arrived less than half a minute apart. After securing a supply of honey, they departed in the same manner that they did before. In less than five minutes, the two had returned. One of these, after arriving at disc one, left it and went to disc two and obtained honey. These three bees were watched carefully for half an hour. In that time fourteen visits were made to the red discs; eleven to disc one and three to disc two. No visits were made to disc three. On two occasions three and on two other occasions two bees were on disc one at the same time. On each of these occasions, before departing for the hive, the bees always explored the neighborhood of the disc.

For the half hour or more that I was conducting this experiment, I was too much occupied to pay any attention to the discs on the other weed. I now watched them continuously for fifteen minutes. Although the weed was alive with bees, no bee visited the red discs. Had any of the bees discovered those discs and had they begun to collect honey from them, some of them should have made a return trip while I was watching. It is reasonable to conclude that no bees had visited them.

Thus, although over a dozen discs, well supplied with honey, were exposed, on weeds that were alive with bees, for fully three hours, yet only three bees visited those discs and their visits were confined to two of them![1]

EXPERIMENT 7.—*On leaving for home, all of the discs were removed from the field except the three from which the bees were collecting honey. On those discs I placed all of the honey that they would hold.* This was done hoping that, before dark, other bees, by imitation, would learn to collect honey from those discs.

[1] I have tried this same type of experiments at other times. Although considerable time always elapsed before the discs were attended to by the bees, yet the time was seldom as long as this.

Series II. (July 13, 8 A.M.)

On my arrival at the field this morning, I noticed that all of the honey had been removed from the discs; and, in two cases, that much of the color had been removed in spots. It looked as though the bees had attempted to carry off even the paper that had been saturated with honey.

Experiment 8.—*Among the branches of the same plants of melilotus from which, yesterday, a few bees learned to collect honey, I placed six red and six blue discs. Two of the red discs (1, 4) and one of the blue were attached to the tops of rods five feet high (the height of the weeds), the others were pinned, at different levels, to the branches of the weed. In the center of each red disc, honey was placed. The red discs were numbered from 1 to 6, the blue from 7 to 12. There was no honey on the blue discs.*

Almost immediately a bee alighted on disc one. These discs were watched continuously for a little less than a half hour. During that time no bees visited the blue discs, but they made thirty-nine visits[1] to the red discs. These visits were distributed as follows: disc one, seven; disc two, two; disc three, nine; disc four, seven; disc five, six; disc six, eight. The bees visited the discs on the rods (1, 4) just as readily as they did those attached to the weeds; they visited those high up (1, 2, 4, 6) just as frequently as they did those low down (3, 5). Whenever a bee was ready to depart for the hive, it always made, in the manner already described, a careful orienting flight. From now on the bees began to visit the red discs in such large numbers that it was impossible to keep an accurate record of the number of visits Sometimes as many as ten bees would visit the same disc at the same time.[2] The significance of this marked change in the behavior of the bees will be discussed later.

Experiment 9.—*I selected a red disc from which four bees had been collecting honey and, while the bees were away, placed it about six inches lower on the plant. In its place I placed a blue disc. The blue disc did not have any honey on it.*

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[1] In all of these experiments, whenever a bee alighted on one of my artifacts, it was counted a visit, whether it was the arrival of a new bee or a return visit of a former visitor.

[2] When these experiments were first planned, it was my intention to mark each bee that participated; but, at this stage of the work, I realized that such a procedure would be impracticable and my paint and brush were put away.

The bees that arrived at the weed in the vicinity of the blue disc dropped at once to the red disc, without even pausing before the blue.

EXPERIMENT 10.—*I placed three more rods in the midst of the same weed; on two of them I placed blue discs without honey, and on the other one a red disc with honey.*

At the end of fifteen minutes, all of the red discs were being visited by numerous bees; none of the blue discs were being visited.

EXPERIMENT 11.—*Two rods were placed so near each other that a space of not more than two centimeters separated the discs. One disc was red and the other blue. The red disc was supplied with honey, the blue was not. After they had been in one position for fifteen minutes, the red disc was placed where the blue had been and the blue placed in the place from which the red had been taken.*

During the few minutes that these discs were under observation, many bees visited the red disc; on one occasion, three bees were obtaining honey from it at the same time. Only one bee visited the blue, and, evidently, she was not foraging for honey. She spent at least ten minutes on the disc and most of that time was spent in one place. A part of the time she was rubbing her legs against the edge of the disc, the remainder she seemed to be simply resting.

EXPERIMENT 12.—*Five centimeters from a red disc containing honey, I placed a blue disc containing honey.*

During the ten minutes that these discs were watched, twelve visits were made to the red disc and only one to the blue. On three different occasions, there were three bees on the red disc at the same time. It was at the close of the ten minutes that the first bee visited the blue disc.

Just as soon as the bee had imbibed the honey and left the blue disc, the disc was replaced with a blue disc that was not supplied with honey.

During the next five minutes, five visits were made to the red and none to the blue. One bee hovered momentarily above the blue and then went to the red.

EXPERIMENT 13.—*In a different place from that where experiment twelve was performed, I arranged, close together on rods, one blue* *and two red discs. On the blue disc and on one of the red discs honey was placed.*

During the time these discs were under continuous observation, fifteen visits were made to the red disc that was supplied with honey, one bee alighted on the red disc that did not bear honey, and three bees alighted on the blue disc. These three bees visited the blue disc at the same time; one bee alighted on the disc, and then almost immediately the other two followed.

While the three bees just mentioned were on the blue disc, the rod supporting that disc was gently removed to a portion of the melilotus patch that did not contain any of my experimental discs.

One by one, the bees made a careful orienting flight and then flew away. These discs were no longer kept under continuous observation; but, at regular intervals, they were visited and the honey replenished. On those occasions I would watch each disc for about five minutes. On each trip I found three bees visiting the blue disc. The disc might be free from bees when I arrived; in a short time, however, three bees would arrive. They did not arrive simultaneously; but, before the first arrival had left two more would be there. I therefore concluded that the same three bees that discovered the honey on the blue disc had continued to visit it, and that no other bees had grasped the significance of that blue disc. (Unfortunately, for the reason mentioned above, these bees were not marked and one cannot be absolutely certain of their identity; but, from a knowledge of the habits of bees when foraging and of the time required to make a trip to the hive, I feel certain that they were the same bees.)

EXPERIMENT 14.—*While several bees were collecting honey from one of the red discs that capped one of my rods, the rod was gently carried fifteen feet in the direction of the hive and erected in another patch of melilotus.*

One by one, the bees made a careful orienting flight and then flew away. In a short time they had returned. Often eight or ten bees would be on the disc at the same time. While I was taking my notes, some of the bees hovered within a short distance of the small pad (13 × 8 cm.) on which I was writing, as though they were examining it. From now on this behavior was common.

EXPERIMENT 15.—*While ten bees were foraging on the red disc* *used in experiment fourteen, the rod was gently carried fifteen feet nearer the hive and erected in a place that was free from tall weeds; even the grass had been cropped short by a horse that had been grazing there.*

The bees, on leaving, hovered about the disc a long time, even examining the cork to which the disc was pinned. Then, after describing a shallow spiral, they flew away. In a short time six had returned to this disc. On her return, each bee flew to the cork first and then to the top of the disc. I was now forced to leave the experiment for half an hour. On my return, the disc was being visited by numerous bees. During the five minutes that I watched it, twenty-six visits were made to it. There were always from six to ten bees on the disc.

EXPERIMENT 16. *From the melilotus weed in which the first experiments of this series were performed, I gently removed a rod which was capped with a red disc upon which ten bees were foraging and erected it fifteen feet further away from the hive, in another patch of white sweet clover.*

The bees on leaving made a careful orienting flight. I left the disc for about twenty minutes. On my return, I found seven bees resting on the disc and imbibing honey.

EXPERIMENT 17.—*At that end of the field which was most distant from the hive, and at about fifty yards from the weeds in which the first experiments of this series were performed, there was a large patch of the same plants, from the racemes of which numerous bees were collecting honey. In the midst of these weeds, but well exposed, I placed a red disc upon which I had poured some honey.*

I remained by this rod for twenty minutes, but no bee approached it. At intervals of ten minutes, I made six visits to this disc. Each time I remained five minutes. At no time did I find any bees visiting the disc.

EXPERIMENT 18.—*In a space free from tall weeds, about thirty yards nearer the hive than the weed in which the first experiments of this series were performed, I erected one of my five foot rods and on its top placed a red disc well supplied with honey.*

At intervals of ten minutes, I made four visits to this disc. The first time I found one bee on the disc; the second time, three; the third time, two, and the fourth, four.

At this stage, I removed all of the discs from the field except the one used in this experiment.

At intervals of ten minutes, I made two additional visits to this disc. On the first trip I found ten bees on the disc; on the second trip, I found eight.

On leaving for home all of the discs were removed from the field.

Series III. (July 14, 7:30 A.M.)

Apparatus.—The cornucopias used in this series of experiments were made in the following manner: A piece of cardboard, colored on both sides, was cut the shape and dimensions shown in Fig. 1. It was folded along the dotted lines and the flaps fastened where they lapped. About one centimeter of the apex of the cone was bent over and fastened. When finished, each cornucopia was nine centimeters long, with an elliptical lip six centimeters wide and three centimeters high. The lip was used for attaching the cornucopia to some support. Incidentally it furnished a platform for the bees. Some of the cornucopias were red and some were green.

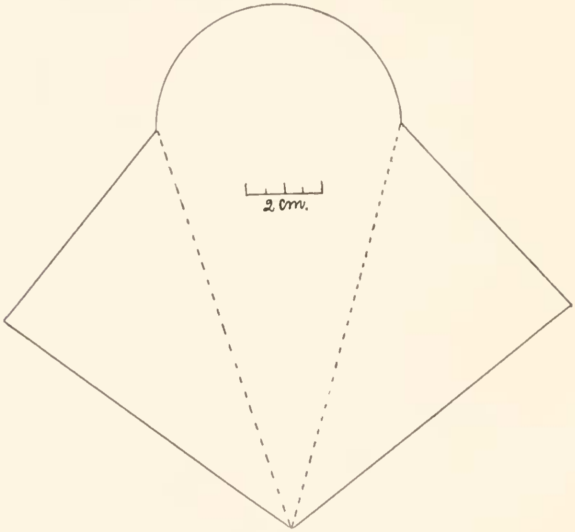

FIG. 1.

EXPERIMENT 19.—*In the weeds that were the seat of the experiments of yesterday, several cornucopias were arranged at different* *levels. Half of these were red and half were green and they were arranged in pairs, one red and one green constituting a pair. The openings of both members of a pair faced in the same direction. All of these cornucopias were attached to parts of the weed. The red ones were supplied with honey, but the green were not.*

Immediately after I had pinned the first red cornucopia to the bush, a bee entered it and began to collect the honey. Two of these pairs were kept under observation for nearly an hour. During that time numerous bees entered the red cornucopias, but not a bee entered the green ones.

EXPERIMENT 20. *Five red cornucopias, each containing honey, were arranged on rods in the following manner: A, in a patch of melilotus about fifty yards further from the hive than the weeds that were the site of experiment nineteen; B, C, D, arranged in a line extending from the patch that was the site of experiment nineteen towards the hive, B twenty feet from the patch, C thirty-five feet, and D fifty feet; E sixty yards from the same patch mentioned above in a line which, at the experiment patch, made an angle of 45° with the line containing B, C, and D. Each of these cornucopias was visited once every twenty minutes, at which times it was watched closely for three minutes. The number of bees in the cornucopia when I arrived were counted, the number to arrive after I did were noted and the sum of the two numbers recorded.*

The results of the above experiment are recorded in the following table:

	Name of the Cornucopia.				
	A	B	C	D	E
Number of bees observed on trip 1	0	1	7	1	0
Number of bees observed on trip 2	0	8	2	8	0
Number of bees observed on trip 3	0	12	6	6	0
Number of bees observed on trip 4	1[1]	21	13	11	2[1]
Number of bees observed on trip 5	1	X	12	16	1
Number of bees observed on trip 6	0	X	X	X	0
Number of bees observed on trip 7[2]	1[3]	21	X	X	6

[1] Before leaving the bee made a careful orienting flight. She examined the cornucopia on all sides several times, reentered it three times, and, after describing a corkscrew curve, flew away to the hive.

[2] There was an interval of an hour between trip six and trip seven.

[3] Two other bees hovered near, but did not enter. The one that entered left immediately. The cornucopia was swarming with ants.

X means that the bees were so numerous that it was impossible to make an accurate count.

Experiment 21.—*Side by side, on one of the branches of the melilotus weed upon which most of these experiments were conducted, I arranged a red and a green cornucopia, and placed honey in each.*

During the first five minutes that these were under observation twenty-five bees entered the red cornucopia and three the green.

Experiment 22. *On rods erected in the open space between the experiment weed and the hive, and about three feet from disc C of experiment 20, a red and a green cornucopia were arranged side by side. Each contained honey.*

During the first five minutes that these cornucopias were watched, sixteen bees entered the red cornucopia and four the green.

Experiment 23.—*The green cornucopia of experiment 22 was replaced by a red cornucopia which did not contain, and never had contained, honey. This placed two red cornucopias side by side, one containing honey and the other empty.*

During the five minutes that these cornucopias were under observation, so many bees entered the red cornucopia which contained honey that it was impossible to count them; five entered the empty red cornucopia.

The empty red cornucopia was now placed where the one containing honey had been and the one containing honey placed in its stead.

During the first five minutes that they were observed, so many bees entered the cornucopia which contained honey that it was impossible to count them; many bees hovered around the empty cornucopia, but none entered.

The cornucopia that contained the honey was removed, the bees shaken out, and the cornucopia put out of sight. This left an empty red cornucopia in a part of the field which, for more than half an hour, had contained at least one red cornucopia which was well supplied with honey.

At first the bees circled around the cornucopia, presently one entered and then left immediately. Within ten minutes twenty-five bees had entered the cornucopia. (This does not mean twenty-five different bees, for the same bee entered more than once and was counted each time.) At first each bee left as soon as she had reached the inner depths of the cornucopia. Soon, however, the bees began to enter so rapidly and in such large numbers that it was impossible for those that had reached the inner depths to leave without a struggle; and, in less than thirty minutes, the cornucopia was packed almost full of struggling bees, and numerous others were hovering around the mouth, seeking a place to enter.

EXPERIMENT 24.—*Ever since the beginning of this series of experiments, the red cornucopias on the melilotus had been kept well supplied with honey. At this time the weed contained eight red cornucopias and an equal number of empty green ones. Into the upper portion of this weed, I placed an empty red cornucopia.*

During the five minutes that this cornucopia was watched, twelve bees entered, one at a time, tarried a moment and then left.

EXPERIMENT 25.—*In the open, three feet from the empty red cornucopia of experiment 23, I placed, on a rod, an empty green cornucopia. In this place, earlier in the morning, there had been a red cornucopia well supplied with honey.*

During the ten minutes that this cornucopia was observed, many bees hovered around it; one alighted on the front platform, but none entered.

All of the cornucopias were removed from the field except the empty red cornucopia of experiment 23 and the empty green one of this experiment. This left only two cornucopias in the field; one red and one green, neither of which had ever contained honey.

This green cornucopia was watched continuously for ten minutes. During that time many bees hovered around the green cornucopia; two alighted on the front platform, and one entered. At the close of the ten minutes, I walked over to the empty red cornucopia and found it almost full of struggling bees, and numerous other bees were hovering around the entrance seeking admittance.

Series IV. (July 15, 3 P.M.)

This series of experiments was conducted with special cardboard boxes; each consisting of a rectangular outer case (8 × 5.2 × 2.5 cm.) with a porch-like extension in front and open ends, into which there was shoved, from the rear, a cardboard tray 5.5 × 5 × 2.4 cm. Near one side of the front end of this tray an entrance was made. In most cases this entrance was a rectangular opening 2 × 1.2 cm.; in a special case it was circular and 1.5

cm. in diameter. The tray was shoved in from the rear until its rear end was just inside of the rear edge of the outer case.

In constructing the outer case, a piece of cardboard was cut the shape and dimensions of figure two, folded along the dotted lines and glued where the sides overlap. In constructing the inner tray, a piece of cardboard was cut the shape and dimensions of figure three, folded along the dotted lines and glued, by the flaps, to the inner portions of the adjacent sides.

EXPERIMENT 26.—*In the same weed that has been the site of most of these experiments, I placed one green and two red boxes. The green box was placed near one of the red boxes. The red boxes contained honey, the green was empty.*

It had been raining all morning and it was still quite cloudy, and only a few bees were afield. About five minutes after the beginning of the experiment a bee noticed one of the red boxes. She examined it carefully from all sides, found the entrance and entered. Soon after she entered, a heavy shower of rain began to fall and I took shelter under a tree. On my return (about fifteen minutes later) I found a large digger wasp in box number one and bees visiting red box number two. As long as that wasp remained in box number one, no bee would enter it. During the time that these boxes were under continuous observation, fifteen bees visited red box number one and twenty-eight visited red box number two. Towards the close of this experi-

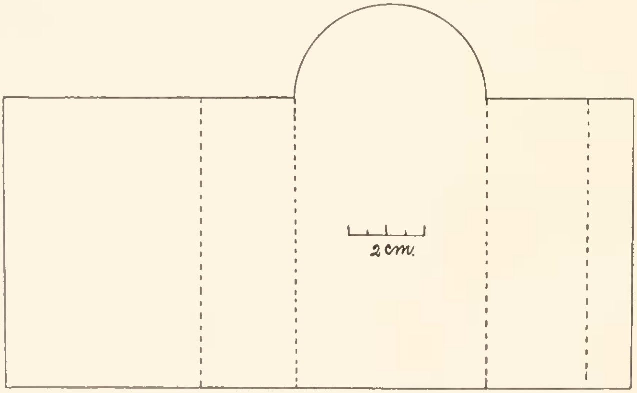

FIG. 2.

ment, some of the bees would fly into the tray without first alighting on the portico. Not once was the green box visited by a bee.

EXPERIMENT 27.—*On my return after the rain mentioned in experiment 26, a watch-glass, seven centimeters in diameter, containing honey, was placed on the ground within a foot of the weed in which the boxes mentioned in experiment 26 were located. It was left uncovered; but, on such a cloudy afternoon, it was not conspicuous.*

During the time that I was watching experiment 26, no attention was paid to the watch-glass. At the close of that experiment the watch-glass was observed continuously for ten minutes. During that time not a single bee visited the watch-glass. Since the trip to the hive required less than five minutes, any bee that had succeeded in finding this watch-glass full of honey would have made at least one visit while I was watching; hence it is logical to conclude that no bee had visited it.

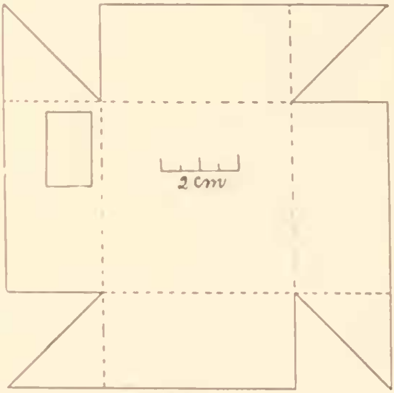

FIG. 3.

At this point rain caused a recess until eight A. M., July 16. On leaving for home, all of the boxes were removed from the weeds and the watch-glass taken from the ground.

EXPERIMENT 28.—*Side by side, in the same weed that has been the seat of the majority of these experiments, I placed two red experiment boxes; one contained honey and the other was empty.*

As soon as I appeared on the scene, the bees began to hover about me, and, before I could pin the red box with its supply of honey to the weed, a bee had entered its tray. In a few minutes so many bees were visiting the red box that contained the honey that it was impossible to count them. Frequently bees would enter the empty red box, but they would not tarry long.

EXPERIMENT 29.—*In the open space between the experiment weed* *and the hive, I arranged, on poles, about ten centimeters apart, three red boxes containing honey, one empty red box and one empty green box. The arrangement of the boxes in the group was altered once in ten minutes.*

These boxes were under continuous observation for about an hour. Immediately the bees began to visit the boxes that contained honey in such large numbers that it was impossible to count them. Occasionally a bee would enter the empty red box and frequently they would hover in front of the entrance to its tray. No bee entered the green box, although occasionally a bee would alight on its top and pause long enough to clean its legs on an edge of it, and frequently one would pause a moment before some portion of the box. Whenever the honey was exhausted from one of the trays, the number of visitors would drop off. Seldom would a bee pass through the entrance; frequently one would hover momentarily before the door and then pass on. As soon as I had replenished the honey, the bees would begin to revisit it.

Experiment 30.—*After the above experiment had been under way for an hour, all of the boxes were removed from the field, except the empty red and the empty green box of experiment 29.*

Immediately the bees began to enter the red box more frequently than they had hitherto; as soon as one got well inside, it would leave. After a lapse of a few minutes, the bees began to rush for the entrance in such large numbers that those that had entered and wanted to leave could not do so without a struggle. As a result the tray and the portico were crowded with struggling bees, and numerous others were hovering about the entrance, seeking admittance.

At first no bees entered the green box, although many circled about it. After a lapse of ten minutes a few began to enter. During the period of observation, ten were noticed to enter it and leave immediately, and about twice that number were noticed to alight in the portico.

Experiment 31.—*Standing about three feet from the above boxes, I held, in my hand, a red box containing honey.*

Immediately a few bees approached and entered the box. I held the box in my hand for about five minutes. Throughout that period there were from one to four bees inside of the box all the time.

EXPERIMENT 32.—*Standing in the same place mentioned in experiment 31, I held an empty red box in my hand. This box had never contained honey.*

Several bees approached and hovered around the box, and two entered.

INTERPRETATION OF THE EXPERIMENTS.

Vision or smell or some combination of the two are instrumental in guiding insects to flowers. Lately some observers have suggested that vision plays no part in this behavior; indeed, it is claimed that it is not even olfactory perceptions, but odors acting reflexly that lead insects to flowers. No one who has made observations for himself would think of claiming that smell plays no rôle in insect behavior; but, the experiments described above show conclusively that odors, acting reflexly, do not lead bees to flowers. In localities where bees are not accustomed to obtain honey from anything but flowers, honey may be placed on small discs (Ex. 1, 17), or in open vessels (Ex. 20 *A* and *B*, 27) and left exposed for a long time without being responded to by the bees. Bees, captured in bottles and turned loose upon discs that are well supplied with honey, will usually depart without paying any attention to the honey (Ex. 2). If a flower upon which such a bee is foraging is so manipulated as to bring the bee in close proximity to the honey of one of those discs, she will usually depart without responding to either the honey or the disc (Ex. 3, 4). It is incredible that an odor of honey too weak to attract bees a few millimeters off is able to entice them from several meters in the air. Then, too, each of my discs contained more than a thousand times as much honey as any one of those flowers did nectar, and the cornucopias that I used contained more than the discs; yet the bees passed by the feast of honey prepared for them to sip the meager supply of nectar stored in the neighboring small flowers. Such behavior would be impossible if their movements were controlled by the honey-odor acting reflexly. To claim that the nectar of the flower has a greater attractive power than the honey would be illogical for: 1st, honey is concentrated nectar; and 2nd, these same bees, after they have learned to collect honey from objects other than flowers, will visit such objects as soon as they are attached to the support (Ex. 8, 19), at times they will even enter them while they are being attached to the support (Ex. 28), or they may even enter such an object while held in the hand (Ex. 31, 32).

If a set of bees has become accustomed to collect honey from artifacts, and paper discs, arranged in pairs of one red and one blue (or discs of any other two colors) are scattered on weeds or placed on weed-high rods, and honey is placed on the discs of one color and none placed on those of the other color, the bees will make regular visits to the color that bears the honey, but will not so respond to the other color (Ex. 8, 9, 10, 11). If these experiments are repeated, using cornucopias (Ex. 19) or boxes with small openings (Ex. 29) in place of the discs, the results will be the same. If bees have become accustomed to collect honey from an artifact of a certain color, and empty artifacts of the same kind and color are placed along side of those that contain honey, many of the bees will enter those artifacts that never have contained honey; empty artifacts of a different color are not responded to in that manner (Ex. 23, 24, 28, 29). After bees have, for a long time, been collecting honey from artifacts of a certain color, if all the artifacts be removed from the field except two that never have contained honey, but one of which is the color of the artifacts from which the bees have been collecting honey, numerous bees will flock into the empty artifact of the same color as those from which the bees have been foraging; but none, or nearly none, will visit the other artifact (Ex. 25, 30). When bees have become accustomed to collecting honey from other sources than flowers, if receptacles of two different colors are placed on a bush, all of one color containing honey and all of the other color being empty, and if, after the bees have been busy for a long time, honey is placed in one of the artifacts of the color that has been empty all along, it will remain on the bush some time before it will be visited by any bees (Ex. 12, 13, 21, 22). All of the facts recorded in this paragraph indicate that the behavior of foraging bees is influenced by colors.

That these bees not only respond to colors, but that they are capable of recognizing them at a distance is evidenced by the following facts: (1) If, in an open space between their hive and a number of artifacts from which the bees are collecting honey, you place an artifact of the same kind and color and supply it with honey, it will be visited by bees almost immediately (Ex. 18, 29). (2) When bees are collecting honey from red artifacts situated in two different stations, one of which is much nearer the hive than the other and beneath the line of flight of the bees collecting from the more distant station, if all of the artifacts are removed from the more distant station, immediately the number of visitors to the other station is much increased (Ex. 18). This was the case even when the artifact in the nearer station did not contain honey (Ex. 30). Empty artifacts of another color than the honey-bearing color were not responded to in this manner (Ex. 30.).

On leaving one of these artifacts the bee usually made an orienting flight. On the first few visits this was thoroughly done. Facing the artifact and keeping about one centimeter from its surface, she would sidle, in a zigzag line, around the structure two or more times and occasionally reënter it one or more times. Then she would describe one or more spirals, pausing at certain places in the environment as though examining landmarks. In some of the cases I was, apparently, one of the objects thus scrutinized. In harmony with her other behavior, it seems plausbile to interpret this as an act by which memory pictures of the environment are formed.

How minute are the details that bees observe I am not prepared to say; but that they do observe details is indicated by the following observations. (1) In the boxes used in the experiments of Series IV., the trays of the boxes used were entered, from the portico, by means of an eccentric opening. In most of the boxes this doorway was rectangular, in others it was circular. When a bee first approached one of these boxes, she had to search for the entrance. After a few trips she would land on the portico directly in front of the entrance, and, in some cases, she would fly into the tray without even pausing on the portico. (2) For about an hour bees had been collecting honey from some red artifacts. It seemed that nearly all of the bees that visited that part of the field were collecting from those artifacts. Alongside one of the red boxes I placed a red box on the sides and top of which I had pasted bits of white paper. This gave a box with red front and spotted sides. Into this box I placed some honey. The bees that approached this box from the front always entered immediately; the majority of those that approached the sides paused a moment, then went to the nearest red box.

Whether this is a true color vision or simply a greyness discrimination is no easy question to answer; indeed, from our viewpoint, it does not seem an important one. If what to us is red and green appears to the bees as two distinct sensations, as a factor for controlling behavior, it will have the same value to the bee whether it is a red-green discrimination or a grey-grey discrimination. However, the following line of reasoning has led me to believe this a case of true color-vision. Bees that had learned to respond to red boxes in the shadow of the weeds would respond, without hesitation, to similar boxes placed in the sunshine. They responded to the boxes when the sun was shining brightly just as readily as they did when a dark cloud hid the face of the sun. The brightness content of a body in the bright sunlight is quite unlike the brightness content of the same body when in the shadow of weeds; the brightness content of a body in the sunshine is quite unlike the brightness content of the same body beneath a cloudy sky. The only factor common to all of these cases is redness; hence I feel that, with the bees, it is a case of true color vision.

Although odor as a incitive to reflex actions does not play any part in leading bees to flowers, yet odor as a sensation does. If a large number of bees are collecting honey from a cluster of boxes that are all of the same color and you allow the honey of some of those boxes to become practically exhausted while that in the others is constantly replenished, when the workers approach the boxes that are practically exhausted, they, as a rule, do not pass inside; but, pausing momentarily before the box, pass on to one of those with an abundant store of honey. Replenish the empty tray with honey, and the next bee that approaches that box will enter (Ex. 29). This, to my mind, shows that when a bee approaches a box and finds the honey-odor weak she immediately departs for a box where the honey-odor is stronger.

These experiments prove that, to the bee, my colored discs, my colored cornucopias, and my colored boxes were something more than mere sensations; it seems to me that they were true percepts. To the bees those things had acquired a meaning; those strange red things had come to mean "honey-bearers," and those strange green things and strange blue things had come to mean "not-honey-bearers." Hence, whenever the bees saw the red things, they made the appropriate movements for securing the honey, and when they saw the blue things or the green things they passed on. This explains why, in the experiments of series one, discs six centimeters in diameter and well supplied with honey could remain in the presence of hundreds of bees without being responded to by them; and yet, those same bees, a few days later, when those things had acquired a meaning, would enter my red boxes even before I had had an opportunity to attach them to their supports. In their past experience those things had never acquired a meaning, while the small blossoms of the melilotus had come to mean "honey-bearers"; hence they hastened by the feast that had been prepared for them and rushed for the meager supply of nectar in the blossoms of the white sweet clover.

Although Plateau's conclusions are diametrically opposed to the results of this series of investigations, yet the facts related by him are in accord with them.

While proving that bees have color-vision, these experiments throw no light upon the color preferences of insects. That has not been the purpose of these researches. The aim has been to answer the question, Can bees distinguish colors? The experiments seem to demonstrate that foraging bees have percepts and that two factors which enter into those percepts are color sensations and olfactory sensations.

Summer High School, St. Louis, Mo.,
July 18, 1910.

BIOLOGICAL STUDIES ON CORYMORPHA. IV.[1]

Budding and Fission in Heteromorphic Pieces and the Control of Polarity.

HARRY BEAL TORREY.

The large solitary hydroid *Corymorpha* exhibits the phenomenon of heteromorphosis in forms even more striking than those under which it appears in the related *Tubularia*. At the same time, its normal polarity is in several respects more obviously marked. As against a stem, in *Tubularia*, that presents little or no indication of axial differentiation, the column of *Corymorpha* is divided into several regions sharply characterized by differences in form, structure and function. Its diameter varies, being greatest near the base, which is enveloped, for about one third the total length, in a thin layer of perisarc. Beyond the edge of the latter, the naked ectoderm is thicker, its cells are more narrowly columnar, and there is a marked increase in the number of nematocysts. Within the perisarc is the zone of frustules, or rootlets, that form the holdfast and have been homologized with the stolonal processes of *Tubularia*, although they are far more specialized structures. The proximal extremity, conical in form, is furnished with an amœboid ectoderm, by means of which the polyp creeps about.

Not only in structure does one find evidence of regional differentiation, but in capacity for regeneration as well. A hydranth is replaced after section of the column, with a velocity that decreases with the distance from the distal end of the intact hydroid. The differences in velocity are so slight as to be appreciated with difficulty in the distal half of the column, but are easily recognizable in a comparison of rates of regeneration in distal and proximal thirds. Furthermore, heteromorphosis, though it may occur after section of the column even below the frustular zone, in the extreme basal region, is most frequent in pieces cut from the column in its distal half.

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[1] Contribution 32 from the Laboratory of the Marine Biological Association of San Diego. Preceding numbers of the Biological Studies on Corymorpha have appeared as follows: I., *C. palma* and Environment, *J. E. Z.*, 1 (1904), p. 395; II., The Development of *C. palma* from the Egg, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 253; III., Regeneration of Hydranth and Holdfast, *ibid.*, 6 (1910), p. 205.

At the very beginning of my observations on the regeneration of *Corymorpha*, I was struck with two facts: (1) that a segment from the distal half of the column and including the hydranth, does not develop a hydranth at the proximal end until the original hydranth is removed; (2) that when the original hydranth has been removed, the proximal hydranth develops, under normal conditions, more slowly than the distal—another indication, it may be mentioned, in passing, of the initial polarization of the column.

These facts suggested the possibility that, by delaying the development of the distal hydranth on a regenerating piece until the proximal hydranth should have reached an advanced stage of development, the initial polarization might be completely reversed. Accordingly, in the summer of 1902, I performed the following experiment.[1] A segment was cut from the distal half of an average polyp (Fig. 1, *A*). It was then inverted, and the distal cut surface held against the glass bottom of the aquarium by the weight of a steel needle through the distal region (Fig. 1, *B*). The proximal end was free and the stem vertical. At the end of three days, a hydranth flourished at *p*, though there was sign neither of tentacles nor frustules at *d*. The needle was removed. Two days later a small hydranth had appeared at *d* (Fig. 1, *C*), with three distal and four proximal tentacles, somewhat irregularly arranged, probably owing to the wound left by the needle and to other adverse conditions to which that end may have been subjected when pressed against the substratum.

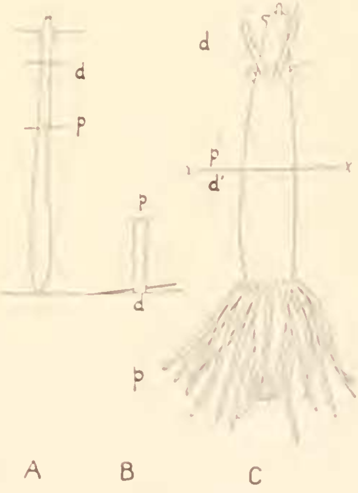

FIG. 1.

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[1] All the regenerations noted in this paper occurred under starvation conditions.

It will be noted that while a distal hydranth failed to develop in contact with the substratum, it soon appeared when freed from this contact, in spite of the presence of the large promixal hydranth. On the supposition, however, that the result may not have fully indicated the real state of affairs in the heteromorphic segment, the latter was sectioned at the level x. In two days frustules were appearing at p'; the original polarity of this portion of the column was preserved. But frustules were also appearing at d', and two days later, were unmistakably defined. *In this latter region, therefore, the original polarity was reversed; on a segment of a given polyp, not only had hydranth appeared in the customary position of holdfast, but holdfast had appeared in the customary position of hydranth.*

The outcome of this experiment recalls the reversal of polarity which Loeb later obtained in *Tubularia crocea* when, after accelerating the development of the proximal hydranth by inhibiting the development of the distal, he cut a segment just distal to the proximal hydranth and found that a proximal was now produced more rapidly than a distal hydranth.[1] Morgan and Stevens obtained a similar result on *T. marina* although the polarity of the stem was reversed for but a very short distance from the proximal end in this species.[2]

II.

The suggestion, coming from the above experiment with *Corymorpha*, that section of the column between the hydranths merely disclosed a reversed polarity that already existed but was not, under the conditions, expressed in structural differentiation, led to a number of similar experiments which showed that the original result was in no sense exceptional. I will consider three series of these experiments.

In the first, nine heteromorphic pieces were sectioned at different levels to determine the extent to which each hydranth might control the intermediate region in regeneration. In no. 1, the distal hydranth was removed by a cut immediately below it

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[1] *Pflüger's Arch.*, 102 (1904), p. 152; trans. in *Univ. Calif. Publ. Physiol.*, 1 (1904), p. 151.

[2] *J. E. Z.*, 1 (1904), p. 559.

(Fig. 2, *a*); it was replaced by another hydranth that at the end of ten days was in the condition shown in Fig. 2, *b*. No. 2 was a similar case. In neither of these cases was reversal exhibited. No. 3 died. No. 4 is represented in Fig. 3, the proximal hydranth being much less developed than the distal, and the plane of section passing near but not immediately distal to it. In four days both segments possessed frustules; the proximal segment had, accordingly, reversed completely. No. 5 (Fig. 4) exhibited another case of complete reversal in the proximal segment; both segments were as shown in Fig. 5 at the end of five days. No. 6 was a similar case. No. 7, cut when in the condition shown in Fig. 6, appeared, five days later, as shown in Fig. 7. No. 8, a similar case, exhibited similarly a complete reversal in five days. No. 9 resembled Fig. 2, *a*; but it was the smaller, *proximal* hydranth that was removed, by a cut immediately distal to it; in seven days a new hydranth was established in its place.

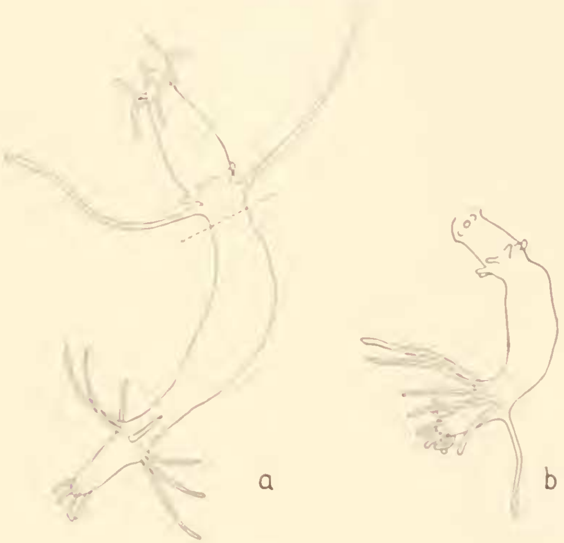

FIG. 2.

According to these results, heteromorphic pieces produce holdfasts at the wound when sectioned approximately midway between the hydranths, but produce hydranths at the wound on the longer pieces in those cases in which either hydranth has been removed by a cut immediately below it.

The second series of experiments involved 14 heteromorphic pieces whose proportions and stage of development are represented in the accompanying diagrams (Fig. 8). These pieces were cut as indicated in the diagrams. Four days later, both parts of *a*, *c*, *d*, *e*, *f*, *h*, *i*, *l*, were attached and possessed frustules; the polarity of one of the pieces in each of these cases, accordingly, was completely reversed. At the same time, both portions of *b* were regenerating as single polyps, one being attached and furnished with frustules, the other being unattached and lacking frustules. Under *g*, four heteromorphic pieces were grouped. Four days

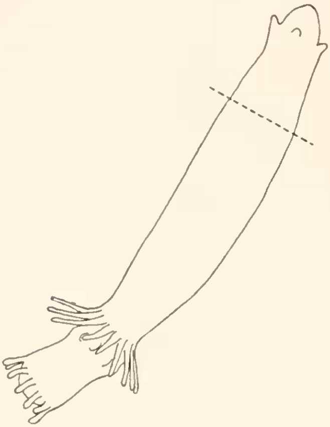

Fig. 3.

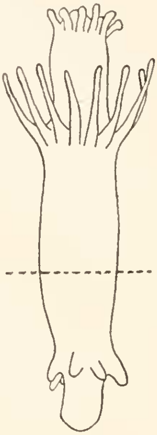

Fig. 4.

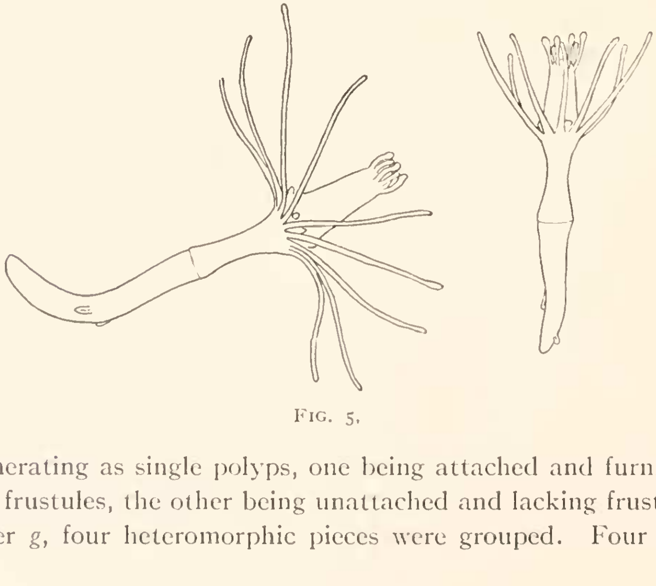

Fig. 5.

after cutting, all were regenerating as single polyps, six being attached and possessing frustules, two being unattached and lacking frustules. Both pieces of *k* were attached, three days after cutting, but owing to accidental neglect, disintegrated before forming frustules.

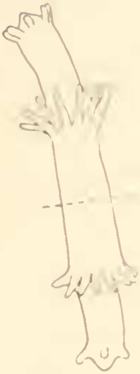

Fig. 6.

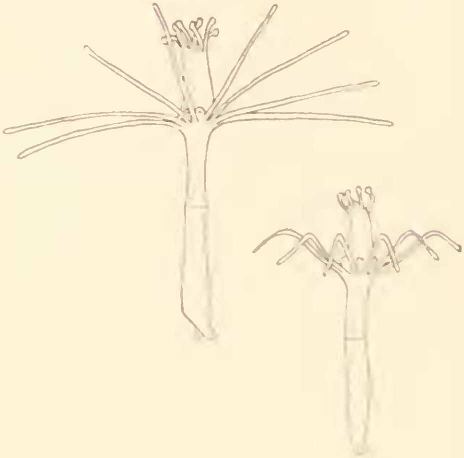

Fig. 7.

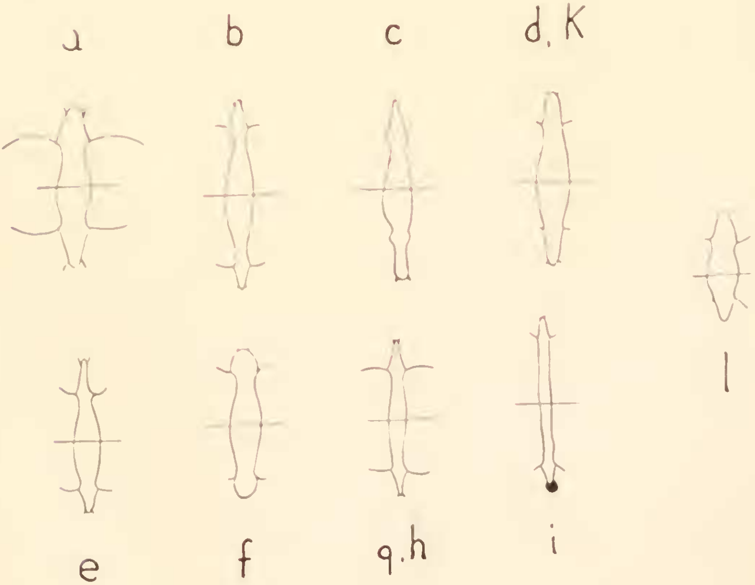

Fig. 8.

It should be noticed especially that all the heteromorphic pieces used in this series were *short*, and none were above medium diameter. *In not a single case, under these conditions, did the polarity fail to reverse in one of the portions into which the heteromorphic pieces were divided.*

The third series shows the importance of this consideration of length. Three heteromorphic pieces, absolutely and relatively much longer than those of the second series, one of them considerably larger than the other two, were sectioned as shown in Fig. 9. Five days after section, all six pieces were heteromorphic which indicated that in none of them, whether distal or proximal, was development at the wound dominated by the conditions, existing at the other end of the piece.

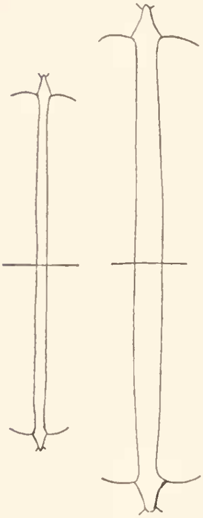

FIG. 9.

The same fact is brought into clear relief by a comparison of the following figures. Of 14 segments representing the distal half of the column of 14 polyps of moderate size, 12 were heteromorphic in 3 days. Of 81 very short segments from several small polyps, only 6, or 7.4 per cent., became heteromorphic. Further, of 13 segments of approximately the same length and diameter as the pieces obtained by cutting the heteromorphic pieces in series 2, 8 became heteromorphic. Of 15 similar segments, 10 became heteromorphic.

Besides these figures, there is a mass of evidence, obtained by repeated experiments on large numbers of individuals, demonstrating that the presence of the original hydranth on a segment of the column inhibits the development of a proximal hydranth.

It is clear, then, in the light of the facts cited in this section, (1) that *reversals of polarity profound enough to effect entire segments of the column as units are readily produced in Corymorpha;* and (2) that *the stage of differentiation at one end of a piece will under certain conditions control differentiation at the other end.* That reversals of polarity, in cases of heteromorphosis, are often shown, by form changes, to affect considerable areas of the column, without aid from the knife, will appear in the following section.

III.

As the first evidence in this direction I may refer at once to certain U-shaped figures formed from heteromorphic segments by the attachment of the latter to the bottom of the aquarium dish by means of adhesive ectoderm developed on one side of the column. Such a case is shown in Fig. 10, which represents the condition of a segment sixteen days after its removal from the distal half of a column. A constriction defines the aboral limits of the regenerating polyps. Frustules are present in two groups, roughly proportional in numbers to the sizes of the polyps to which they belong, and situated in the position normally occupied by frustules in the adult. The smaller polyp approaches more closely to the proportions of the larva. On the following day, this process of fission had been completed, probably by rupture, although the proximal ends of the two polyps were then rounded and smooth without traces of such a process.

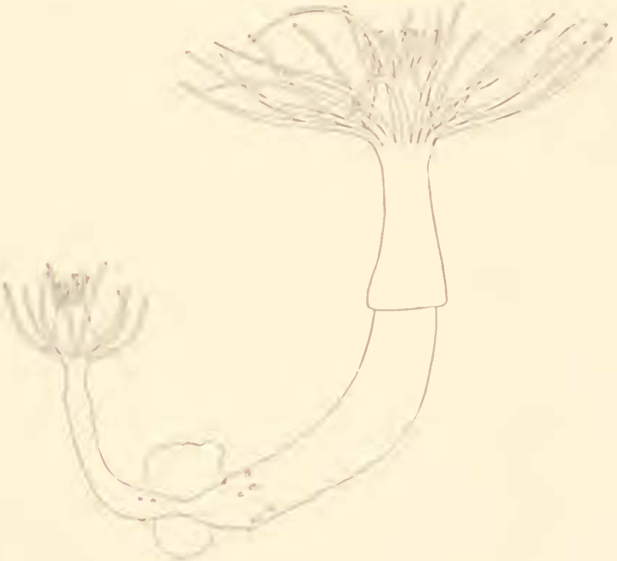

FIG. 10.

Through my failure, after repeated attempts, to obtain many cases of fission of this sort, I found that a necessary element in the process was the adherence of the heteromorphic piece to the substratum. I pointed out some years ago[1] that *Corymorpha* is negatively geotropic, even small fractions of the column reacting with great definiteness. This tendency to bend away from the center of the earth can be effective, however, only when the reacting piece is properly anchored. Pieces free from the substratum never exhibit the reaction. As soon as they are attached, it appears; and U- and Y-shaped figures are formed when pieces are heteromorphic and the point of attachment is between the developing ends.

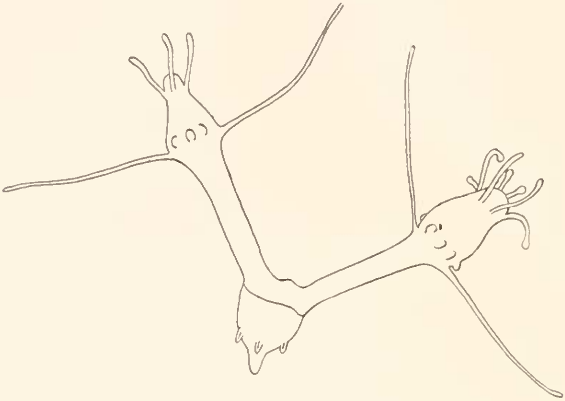

FIG. 11.

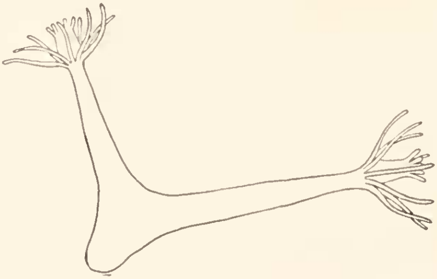

FIG. 12.

Each Y-shaped figure is formed from a straight piece by the development of a protuberance that corresponds to the stem of the Y, and is terminally adhesive (Figs. 11, 12, in both of which the limbs of the Y have become much attenuated during the development). Such protuberances and Y-shaped figures are found in nature only in the most exceptional cases, where they probably arise in response to the same conditions that bring them forth in the laboratory. To present these conditions with sufficient fullness, I must refer briefly to other abnormal forms that have often appeared in neglected aquaria.

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[1] *J. E. Z.*, 1 (1904), p. 395; *Univ. Calif. Publ. Zool.*, 2 (1905), p. 335.

It was often difficult, in warm weather, in the absence of running water, to prevent the growth of bacteria in the dishes in which freshly collected polyps were placed. Under the influence of fermentative changes induced by these conditions, the hydranths would cast their large proximal tentacles and medusiferous peduncles, the distal tentacles would be absorbed, and the column would come rapidly to be surmounted by a more or less rounded mass of tissue that might show two or three knobby irregularities. The columns were affected in a much less degree if at all. In fact it is quite easy to avoid these difficulties altogether by removing the hydranths as soon as the polyps are collected. This fact is doubtless due to the relatively large mass of protoplasm in, and the greater differentiation of the tissues of the hydranths.

Upon the reduction of the hydranths to the knobby masses just mentioned, and the removal of the débris composed of disintegrating tentacles and medusæ with their peduncles, the bacteria would disappear, fermentative processes would lead to the substitution, for the original hydranths, of various monstrous forms—double hydranths, hydranths with double or triple probosces in varying degrees of independence, combinations of hydranths with varying numbers of probosces, etc. These phenomena indicated the breaking up of the original single physiological system into several, the first sign of this multiplication appearing in the irregular form of the terminal mass of tissue. Each irregularity was the center of a budding process. And without laying any emphasis on the manner of its initiation, each budding process may be compared directly with the process by which the stem is produced in the Y-shaped figures we have been considering.

In one important respect these budding processes resemble each other; they stand, namely, for a certain disorganization (how

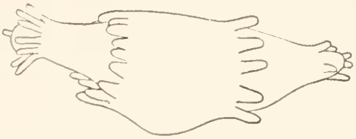

FIG. 13.

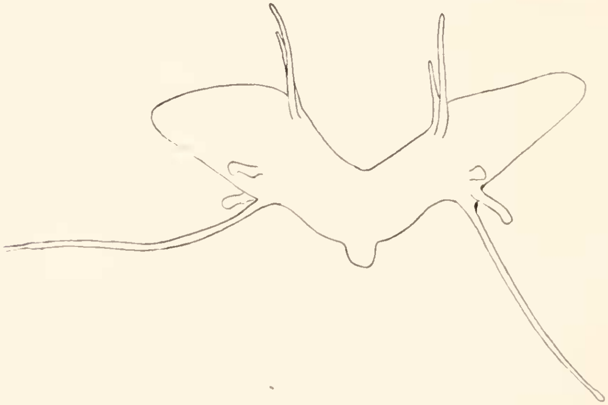

FIG. 14.

produced may, for the time, not detain us) in the original physiological system. In another respect they differ, in that they lead, in the one case, to a hydranth, or part of a hydranth, in the other to a holdfast. This difference is essentially an expression of the different conditions controlling their development, of which the influence of adjacent parts is the chief. The intimacy of this cöordination is obviously a function of the physiological isolation of the parts concerned. It is a conspicuous fact that Y figures are formed almost invariably from short heteromorphic segments; shrunken, starving, slow developing (Fig. 13, which shows eth beginning of a bud) pieces give an especially large proportion of them. Frequently that portion of a segment of the column which lies against the floor of the aquarium puts forth tentacles more slowly than the upper surface. This retardation in development, due probably to diminished supply of oxygen next the substratum, is accompanied by a bending of the segment by means of a contraction of the affected side. It is on the opposite, convex, aspect of the column that the bud develops (Fig. 14, in which tentacles and gonads are only partly drawn), not, therefore, in direct contact with the substratum. And it is significant that, with the rarest exceptions that are referable to exceptional conditions, such buds arising on heteromorphic pieces become holdfasts.

These facts indicate that while the origin of the bud depends upon a degree of disorganization in the original physiological system, its *fate* depends upon a secondary physiological coördination with the hydranths between which it develops. The bud acquires the distinctive character of a holdfast, namely, its adhesiveness, independent of any influence of the substratum. It has been pointed out already that a hydranth at one end of a piece exercises a profound influence upon the differentiation that may occur at the other end, depending on its own stage of development and its distance from that end. In the Y figures, then, there is a developing region between the two hydranths whose differentiation is controlled to some extent by them. An interesting case of the coördination of parts in a short piece developing as a Y figure, is represented in Fig. 15. A new axis at right angles to the original axis has been established. And the orientation of gonads and tentacles—especially the latter—is clearly a resultant of the redistribution of forces correlated with that change.

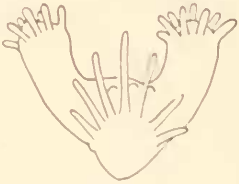

FIG. 15.

Fission may be considered as a special case of budding, depending upon the length of the heteromorphic piece involved. As the piece lengthens the tendency to bud vanishes, until the characteristic of adhesiveness alone remains. This indicates that the region between the two hydranths is still controlled by them, while the constriction and the frustules on either side of it (Fig. 10) mark the increasing effectiveness of their independence—or of the systems of which they form important parts.

It is only on heteromorphic pieces of moderate length, however, that fission of this type has been observed. Just as it was from relatively short heteromorphic pieces only that the reversals described in the previous section were obtained, the proximal elements of the longer pieces failing to produce holdfasts at the wound, so it has been only in the shorter heteromorphic pieces that the column between the hydranths has shown any signs of budding, constriction or frustule formation.

The longer pieces failed to differentiate in these directions, although attempts were made to encourage such developments, as follows:

1. Nine long heteromorphic segments were held against the aquarium bottom by weighted glass needles laid across their middle. One was cut in two in two days; neither portion had developed frustules twenty-four hours later. One was cut in two in three days; both portions had aboral processes, but no frustules developed in the following twenty-four hours. Four escaped from their needles on the third day, and showed no change at the end of another day. One was almost cut through by the needle, and three processes were formed at the wound, but no frustules or adhesiveness at the end of another day. One remained under the needle unchanged for four days, when the experiment terminated with my departure from the laboratory.

2. Discontinuity was produced by ligature on 16 long heteromorphic pieces two days after regeneration had begun. In two days, 3 had broken into two parts. Two days later, 6 more had done so; the next day, 7 more. Seven days after the ligatures were applied, 2 had frustules at the ligatures. Both were contracted and opaque—signs of structural degradation; 3 possessed no frustules at the ligature; on the contrary, the proximal segment of one possessed a hydranth there. All the other ligatured pieces had separated into two portions, 18 in all, of which but 4 were attached aborally and possessed frustules.

The first of these experiments serves to emphasize the feebleness of contact as a formative influence, while the second adds to the evidence that differentiation in the region between heteromorphic hydranths depends in an important degree upon the distance between them, which other things equal, is an index of their control.

That the longer pieces do not show signs of fission, then, is to be attributed, I believe, to the freedom of the intermediate region in each case from the effective control of the physiological systems on either side, that may be conceived as extending over it from opposite directions. Where these systems are near enough together, a new compound system is created, of which a bud may form a part. When farther apart, their disharmony may appear in the phenomnea of fisson.

These statements are obviously very general. Since all short heteromorphic pieces do not either bud or divide by process of fission, there must be a factor still undefined that determines the lack of uniformity. There is no doubt that external agencies can facilitate either budding or fission. Small wounds in the side of the column may lead to a variety of results, including sporadic tentacles, and buds furnished with tentacles or frustules, or neither. In this connection two heteromophic pieces, cut in the middle region, half way through the column, gave the following results. On the longer piece, a narrow bud developed in four days at the wound, with neither frustules nor tentacles. On the shorter piece, frustules developed around a blunter bud at the wound, before attachment took place. In this case, the wound was sufficient to break up the original system existing at that point and initiate a new development. The fact that frustules appeared shows the control of both hydranths on that development.

Budding occurs, however, when there is no sign of local injury from without. And it is difficult to account on this ground alone for the fact that the large majority of buds arise approximately midway between the hydranths. To my mind, far more significant is the fact that buds develop so often on pieces obviously in poor physiological condition *generally*. It is then that the physiological continuity of the piece through the transitional middle region might be expected to be especially affected by disruptive tendencies springing from the antagonism of proximal and distal systems so obviously antagonistic in the fission shown in Fig. 10. In that case, the canals are completely obliterated at the constriction and the tissue is opaque and apparently impoverished. This constricted region is under tension, which probably accounts in part for its form. The tension is produced by the active migration of the two polyps away from each other, in the manner of the opposite halves of an anemone in process of fission by rupture.[1] The initial discontinuity is thus accentuated by the activities of the polyps themselves.

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[1] Torrey, *Univ. Calif. Publ. Zool.*, 1 (1904), p. 211.

IV.

Discontinuity can be established experimentally not only by the knife, which entails a wound, but, as already indicated, by ligature, by the use of which a wound can be avoided and conditions obtained that more nearly approximate those described in the last section.

This method has been used on *Tubularia* by Driesch, Morgan, Loeb, and Morgan and Stevens, and results obtained which are of interest in the present connection. By ligating segments of the stem, not only is the production of aboral (proximal) hydranths assured, but accelerated; and only exceptionally, after much longer periods, is there any development at the ligature itself. Loeb succeeded in showing that the acceleration of the development of the aboral hydranth is an indication of reversed polarity that exhibits a certain stability in regeneration. This is in accord with what I had already observed in *Corymorpha*, where reversals of polarity accomplished without the aid of the ligature are even more marked.

The experiments with ligatures have been repeated so many times on *Tubularia*, that it is hardly necessary for me to refer at present to similar experiments of my own farther than to say that the ligature accelerated the development of the aboral but not of the oral hydranth, and in no case was there any development at the ligature, on either side of it.

In Corymorpha, as in Tubularia, ligatures accelerate the velocity of development at the proximal ends of segments of the column. The fact does not stand out with such dramatic clearness, however, partly because there is greater individual variation in rate of regeneration, partly because the time intervening between the appearance of distal and proximal hydranths is much shorter. That such an acceleration occurs can be shown by an experiment like the following: Segments about 2 cm. long were cut from the distal half of 20 polyps, a ligature being passed tightly around each near its distal end. Segments of similar length were cut from the distal halves of 21 polyps of similar size; these were not ligatured. All were placed together in the same dish. In 28 hours, there were signs of proximal hydranths on 13 ligatured segments, and 14 on non-ligatured segments. The condition of affairs at the end of 56 hours is shown in the following table, in which the serial numbers represent stages in the development arbitrarily selected for purposes of classification. Under each of these appears the number (1) of proximal hydranths in that stage of development on the ligatured segments, (2) of proximal hydranths on non-ligatured segments, and (3) of distal hydranths on non-ligatured segments.

Table I.

Dev. stages	1	2	3	4	5	6	7	Totals.
1. Lig. (pr.)	0	5	3	5	5	1	1	20
2. Non-lig. (pr.)	4	4	5	6	1	1	0	21
3. " (dist.)	3	2	8	4	1	2	1	21

In spite of the individual variation represented by these figures, they *show a tendency in the proximal ends to develop hydranths somewhat more rapidly on the ligatured segments, and almost as rapidly on the latter as the distal hydranths on non-ligatured segments.*

V.

Although *Corymorpha* responds proximally like *Tubularia* when segments of the column are ligatured as above, there is an important difference in its response at the ligature, namely, in the rapidity with which hydranths are formed immediately below it. In *Tubularia*, a hydranth very rarely appears immediately below the ligature, and then only after the lapse of many days. In *Corymorpha*, on the contrary, hydranths form readily and frequently in this position.

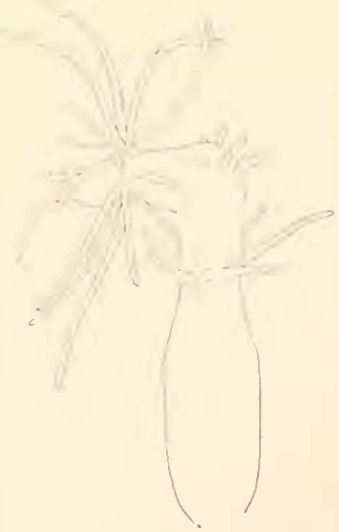

Fig. 16.

That a hydranth should arise immediately below a ligature in *Corymorpha* might be anticipated from the occasional occurrence of such monsters as that shown in Fig. 16, which represents a regenerating segment of the column. The proximal hydranth (below the angle) is not so far along as the distal hydranth, and has apparently developed later, below an interruption in the physiological continuity of the column comparable with what a ligature might produce. In fact, just such cases have been produced experimentally several times. A typical experiment may be recorded, showing incidentally the difficulties that made the number of positive cases so small.

The column of *Corymorpha* is very mobile, capable of considerable changes in length and bulk, and its tissues are very delicate and easily ruptured. So it has been difficult to make ligatures tight enough to interrupt the currents in the canals, as well as possible diffusions through the axial cells, without so weakening the column as to lead to complete rupture in two or three days. This has been accomplished, however, in a number of cases sufficient for the present purpose.

Experiment 1.

April 29, 1910, 3.30 P. M. Sectioned 20 polyps of similar size, about midway of the column, and ligated each just below wound, leaving a small segment of tissue above the ligature.

May 1, 9.30 A. M. Four stumps removed, ligature having come away with terminal button of tissue.

May 2, 5 P. M. Thirteen more removed for similar reason. There are hydranths on these stumps that seem to be too far along, under the conditions, for 31½ hours (*i. e.*, assuming the separation to have occurred immediately after the previous survey of them, which is not probable).

Of the remaining 3, 2 show nothing below the ligature, while the third appears as in the semi-diagrammatic Fig. 17, which bears a striking resemblance to Fig. 16.

May 3, 9.45 A. M. The two hydranths of Fig. 17 have fallen apart.

May 4, 10.00 A. M. Of the 2 stumps showing no development below the ligature of May 2, one (*a*) has now budded a set of tentacles just below the ligature; the other (*b*) as before.

May 6. The tentacles of (*a*) are larger.

May 9. Still no change in (*b*). Exp. abandoned.

In a second experiment, 6 columns were tied as indicated in the diagram (Fig. 18, *a*), after removal of hydranth.

Experiment 2.

April 7, 3.00 P. M. Hydranths removed and columns tied.

April 8, 1.00 P. M. (*a*) Distal ligature and tip fragment (1) broken away in 2 cases. (*b*) The three segments have separated in 1 case. (*c*) 3 cases in original condition.

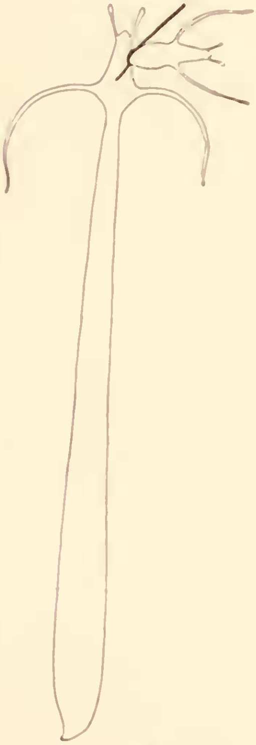

Fig. 17.

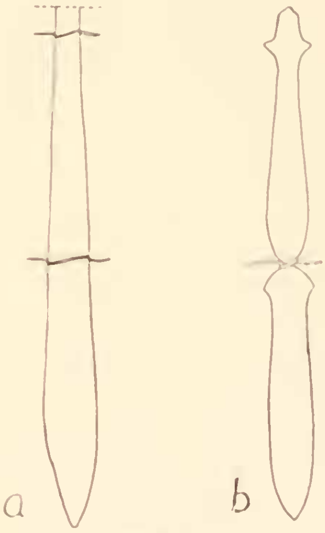

Fig. 18.

April 9, 2.00 P. M. (*a*) First case. Distal hydranth appearing on seg. 2, also on seg. 3 but not so far along. Second case. Distal hydranth appearing on seg. 2; not apparent on seg. 3, (*b*) —. (*c*) First case. The segments have separated while under observation. There are signs of a very slight rupture. and indications of tentacles on seg. 2 distally. Second case. The segments separated at 10.00 A. M. Seg. 2 shows signs of tentacles at both ends. In seg. 3, tentacles are being shadowed forth distally. Third case. The segments have separated, seg. 2 showing faint signs of tentacles distally; seg. 3 in poor shape, removed.

April 10, 9.00 A. M. (*a*) First case. See Fig. 18, *b*. Note tentacles appearing just below ligature. Second case. Similar to first case; tentacles on seg. 3 not quite so far advanced.

April 11, 2.15 P. M. (*a*) Segments in both cases broken apart; exp. abandoned.

Of the six cases considered in this experiment, three show development of hydranths immediately below the ligature.

In a third experiment, the hydranth was removed from a polyp and the column ligatured near the cut and near the base. In three days, tentacles had budded just proximal to the distal ligature, as well as on the small distal segment. The next day, the latter was loosely joined to the segment proximal to it; both segments possessed hydranths with both sets of tentacles.

These results show that *hydranths form readily immediately below the ligature* in experiments like the foregoing.

VI.

The failure of *Tubularia* in such experiments to form a hydranth immediately below the ligature with the facility exhibited by *Corymorpha* is correlated with an important structural difference. The stem of *Tubularia* is encased in a stiff, chitinous layer of perisarc, that offers a certain barrier to the diffusion of gases between cœnosarc and the surrounding medium. The column of *Corymorpha*, furnished with a thin, rudimentary perisarc about its base, is naked for more than half its distal length; in this naked distal region, the ligatures were in all cases located.

When a ligature is passed tightly around a stem of *Tubularia*, the cœnosarc is not only ruptured, but the perisarc, itself intact in most cases, is drawn closely about each end of the cœnosarc thus produced. The result is that, while discontinuity has been established, the cœnosarc remains, as before, separated by the perisarc from the surrounding medium. When a ligature is passed tightly around a column of *Corymorpha*, discontinuity is established without in any way interfering in a comparable degree, if at all, with the diffusion of gases between cœnosarc and sea water. Now, when it is remembered that a discontinuity brought about by a knife in *Tubularia* leads promptly to regeneration at the wound, the fact suggests that *Tubularia* develops with greater difficulty than *Cormyorpha* at the ligature because the cœnosarc receives a smaller supply of oxygen in that region.

This view was tested by the following experiment, in which the factor of contact, which tends to inhibit development orally in *Corymorpha*, was eliminated.

Fifteen pieces, each about 2 cm. long, were cut from fifteen *Tubularia* stems of similar size and condition, just below the hydranth. The distal end of each was inserted in a capillary glass tube (closed at one end by a paraffine plug) into which it fitted easily, without terminal contact, *except with a small quantity of sea water;* the proximal end was free. As a control, 16 similar pieces were cut, both ends remaining free.

Forty-five hours later, the pieces in the tubes had developed nine hydranths on the outer (proximal) ends, nothing on the inner ends. In the control, though fifteen of the sixteen pieces possessed distal hydranths, no proximal hydranths were visible.

Twenty-five hours later still, all the pieces in the tubes possessed proximal hydranths; nothing had developed on the inner (distal) ends. In the control, only eight proximal hydranths were present.

Removed now from the tubes, all the pieces rapidly produced normal hydranths distally.

This experiment seems to establish (1) *that the failure of Tubularia stems to form hydranths, when ligatured, immediately below the ligature, is due to lack of oxygen;* (2) *that encasing the cut distal end of a stem in a glass cap leads to the same acceleration of development of the proximal hydranth as does the presence of a ligature;* and (3) *that accordingly, such acceleration is due to the inhibition of the development of the distal hydranth in the absence of an adequate supply of oxygen, rather than to an interruption of or other change in the course of the circulation in the canals.* The circulation is merely a transportation system, carrying substances favoring development that are removed from the stream by those tissues especially that have free access to oxygen, that is, by the tissues at the open ends of the perisarcal tube. The possibility that oxygen might play such a role in this process of selection was suggested by Loeb,[1] though in a somewhat different form in connection with a discussion of the function of the red pigment granules of the circulation.

The acceleration of proximal development in ligated pieces of *Corymorpha* is connected, not so much with any effect the ligature may have upon the supply of oxygen to the neighboring tissues, as with the inhibitory factors of contact and necrotic change that it introduces. The same factors may play a certain part in the inhibitory effect of the ligature in *Tubularia* also.

It will be remembered that if a piece of *Tubularia* or *Corymorpha* is ligated in the middle, development at the distal wound does not exhibit the acceleration characteristic of development at the proximal wound. This difference is to be explained, I believe, in the following way. Inhibition of development at the ligature on its proximal side hinders the utilization of a certain quantity of substances that would be used up proximally were the ligature not present. That the availability of this material for the distal end does not obviously accelerate the development distally is due, probably, to the initial acceleration of the distal over the proximal development in the absence of the ligature, that is, under conditions of active competition with the proximal end.

VII.

The considerations in the foregoing sections lead to the conclusion that the polarity of *Corymorpha*, of which the initial acceleration just mentioned is one expression, is a product of conditions under which the organism develops, changing as they change; that it is essentially but an inclusive designation for certain phenomena that depend upon both internal and external conditions, all of which can be experimentally controlled. The internal conditions appear in the effect of the continuity of tissue in an intact stem, and the presence of the original hydranth on a segment of stem, both inhibiting the development of a hydranth. The external conditions are represented by oxygen, contact, and necrotic changes such as are produced by ligatures. The first is necessary to all development while the others inhibit the development of the hydranth.

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[1] *Loc. cit.*

With the exception of the third, these are conditions that govern the development, whether embyronic or regenerative and they are just the conditions by whose manipulation the original polarity of the column can be reversed. Since such reversal depends upon an acceleration of proximal relative to distal development, it would seem reasonable to suspect that a local acceleration in the development of the embryo might be the efficient cause of the initial polarity in the individual.

Such a local growth area appears in the embryo at the point which first leaves the egg case and thereupon defines the oral extremity.[1] This extremity may emerge at any point not adherent to the substratum; which qualification indicates that contact limits to a considerable extent the area in which the oral pole must appear. Since the egg case is a certain barrier to the diffusion of oxygen into the egg it is possible that variation in its thickness may be an important factor in determining the position of the oral pole in this area.

Zoölogical Laboratory,
University of California,
August 9, 1910

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[1] Torrey, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 259.

Vol. XIX. *November, 1910.* *No. 6.*

BIOLOGICAL BULLETIN

BIOLOGY OF THE SHAWNEE CAVE SPIDERS.

NORMAN E. McINDOO.

GENERAL INTRODUCTION.

From September 7, 1908, to September 7, 1909, the author held the Speleological Fellowship in Indiana University with residence at the University's Cave Farm three miles east of Mitchell, Ind. The present paper embodies the results of the observations during this time on the habits of spiders in caves and in the laboratory.

The work has been carried on under the direction of Dr. C. H. Eigenmann, professor of zoölogy in Indiana University, to whom I am indebted for suggestions and the loan of his cave literature. I wish to express my thanks to Dr. Charles Zeleny, associate professor of zoölogy, for many helpful suggestions. I am indebted to Dr. Alexander Petrunkevitch for the identification of the specimens; to Dr. A. M. Banta, for the loan of his entire collection of cave spiders, and to Mr. Will Scott, for part of the map of this series of caves. He surveyed and mapped the cave from "1" to "37" or the Upper Dalton (see map, page 321) in the autumn of 1907; the author assisted by Mr. Frank Green surveyed and mapped the Upper Dalton from "37" to "64" in October, 1908.

PREVIOUS WORK.

Published observations on the habits of cave spiders are limited to a few scattered papers which give lists of species and localities.[1] The best studies published are those of Packard[2] and Banta.[3]

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[1] Banks ('06) and Blatchley ('96) give lists of Arachnids from Indiana Caves.

[2] *Mem. Nat. Acad. Sci.*, IV., 1888.

[3] "Fauna of Mayfield's Cave," 1907.

THE SPECIES STUDIED.

Two species of spiders permanently live in the Mitchell caves. One, *Troglohyphantes* (*Willibalda*) *cavernicola* Keyserling, a linyphid[1] is a true cave form; the other, *Meta menardi* Latreille, is an epeiridid and also lives outside of caves. According to Emerton (1902, 190–) the latter arachnid "lives in caves and similar cool and shady places in various parts of this country and also in Europe."

Banta (1907, 65) reports *Erigone infernalis* Key. from the Twin Cave at Mitchell, Ind. I have been unable to find it here, but have taken it in Mayfield's Cave at Bloomington, Ind.

Troglohyphantes has been observed in detail in order to get as far as possible the life history of a typical cave spider. The distribution, food and results of the experiments of *Meta* are given in order to show how an outside form is able to adapt itself to a subterranean life. All notes unless otherwise stated refer to *Troglohyphantes*. The numbers in quotation marks refer to localities in the caves (see maps, pages 321 and 323).

In *Troglohyphantes* there are all degrees of differences in coloration, and in the degeneration of the eyes. The abdomen varies in color from black, dark brown, light to white. The cephalothorax varies from dark, pinkish, light to a white color. The most common combination of colors is a light brown abdomen with a pinkish cephalothorax. The adult females range in length (cephalothorax and abdomen) from 2.4 mm. to 3.7 mm., while the adult males vary from 2.2 mm. to 3 mm.

In the adults the eyes range from eight in number, each with a maximum size of 0.036 mm. in diameter to no external signs of eyes. I have not seen Keyserling's description, but from his figure, which Packard[2] has copied, the eyes are small, and the front middle ones extremely minute.

PHYSICAL ENVIRONMENT.

These spiders are found only in total darkness, where the atmosphere is saturated, and in places suitable for the construction of snares. They are never found where the walls are perpendicular with water covering the entire floor; nor are they found where the walls and floor are dry. Distance from the entrance does not necessarily limit the distribution if the three necessary conditions are present, nor does scarcity of food limit their distribution to a very great degree.

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[1] Banks, '06, classification.

[2] 1888, Plate XV., Fig. 32.

At "2" a few were collected last fall but none has been found since. This is 200 feet from the entrance in total darkness. At "9" a few more than at "2" have been found; at "10" a great many have been taken and "13" was my best collecting ground. This place is 600 feet from the mouth. On several occasions I have gathered two or three dozen individuals here and such wholesale collecting at one place seemed at the time to exhaust the supply, but a month after such a collection had been made, I have been able to duplicate this record. Many have been collected at "14" and at "19." The latter locality is the "Big Room," 1,700 feet from the entrance. Three were seen at "23" and only a few were observed in a branch at "31." This latter place is in total darkness 200 feet from the mouth of the Lower Twin Cave at "32." Not one has been observed between "33" and "36." A few have been collected in a branch at "38" in the Upper Dalton. This location is in total darkness 150 feet from the entrance. A number were taken from branches at "46" which is 1,024 feet from the entrance. This locality was my second best collecting ground, particularly for the cocoons. A few were taken at "50." One was caught in the "Big Room" at "57" and several snares were seen. At "63" almost a mile from the entrance of Upper Dalton, three were observed and several webs were among the rocks at a "cave-in."

Troglohyphantes is also quite abundant in Hamer's Cave one half mile west of these caves. None was found nearer than 300 feet from the entrance.

This archnid shares as wide a distribution as the blind beetle, *Anophthalmus tenus* Horn (Blatchley, '96). It far outnumbers the latter in individuals, but is less evenly distributed. By actual count there are twice as many females as males in all the collections made from the various caves.

Meta is found both in twilight and total darkness. In the Shawnee Cave they are quite abundant from the entrance at "1" to "4." The latter place is over 200 feet from the entrance in total darkness. Several were seen at "6," 100 feet from the mouth. An adult male was observed on the roof at "14"; nine days later, it was seen under a rock on the floor and had constructed a snare. One immature specimen was seen in a branch at "23." At "30," 200 feet from the entrance of Lower Twin, a few live, and at "32" and "33" they are quite numerous.

May 17, I collected eighteen *Metas* and placed them on the north wall by a large pile of rocks at "19" in the "Big Room." August 3, I saw six of them. They had scattered along the wall about 50 feet and among the rock pile. Each had built an orb-web and remains of small diptera were seen in the snares. August 17, after a brief search I saw only three of the eighteen. This absence does not mean that they had died, but that it was impossible to find them.

Locomotion.

This arachnid is very agile and is a good runner. Its long legs and slender body enable it to move from place to place, and to avoid an obstacle with much ease. When not irritated it moves along slowly and gently. When stimulated mechanically, it gives a quick jump, runs and dodges whatever obstacle may be in its way. However, it runs only a few inches and if stimulated a second or third time often drops in an instant and hangs to its web. Sometimes it climbs the web to the place from which it dropped, at other times it lets itself down to the floor and then runs off.

Several were placed on the roof among beads of water and other small obstacles. A pencil was used to stimulate and guide them so that they were obliged to run up against the obstacles. They use the first pair of legs as feelers. These are kept well in advance of the other legs and head so that they can detect an object in front of them the length of the body. While walking or running slowly, they are able to dodge an object every time. If caused to run swiftly, they run against the object, or if the object be a low bead of water, they run over it and pass on.

Webs and Snare.

This spider usually spins a web wherever it goes. It is impossible to see a single thread in the cave with a carbide bicycle lamp unless the thread is coated with water. Quite often on the roof in a slit between two strata of rocks, or in crevices in the walls are found collections of webs which are generally coated with water. These webs do not seem to be of any service to the spider after once spun. They, however, show how it wanders from place to place. It makes a flat sheet-like snare under which it lives. The snare is slightly curved downward and may lie in such a position as to form any angle between 0° and 90° with the floor. The threads are fine and the snare so transparent that it is difficult to see unless it is coated with water. The meshes are so minute that the snare turns water. The snare is supported by many fine threads from the sides, the length of these vary according to the surroundings. When insects fly into the snare they are taken through it by the spider which is on the under side.

Snares are most abundant in the older parts of the caves where the passages are blocked up with clay. At such places the clay banks are more or less perpendicular and the water has washed out many small crevices. Sometimes large blocks of clay tumble down into a heap. Among these heaps of clay blocks and in such crevices as the above the snares are very numerous. In a crevice five inches wide I once saw three snares built one above the other not more than three inches apart. All three were parallel with the floor.

In several places snares are quite conspicuous in the angle between the floor and wall. In such positions the spiders have better routes for travel from one snare to another than in the clay banks.

The third place where snares are found is among the rocks at "cave-ins." The rough cornered rocks appear to form good places for attachment and quite frequently dripping water is present which this species apparently enjoys.

FOOD.

While in captivity I have fed them small gnats, small flies and the spiderlings of *Meta*.

In the caves the following observations were made: At "13" while watching a male who was trying to court a female I saw a white-winged dipteron fly into the snare. In an instant the female seized the insect, then ran back to the male. The latter then ran to the other side of the snare. In a few minutes another white-winged dipteron flew into the snare near the male, and he lost no time in seizing it. Several other times these diptera flew against the snare but failed to be entangled in its meshes. In each case the spiders made a dash for the would-be victim while holding one insect in their mandibles. At an earlier date a spider at this same place was seen eating a white-winged dipteron. On various dates at "14" one was caught eating a myriopod; one, a small gnat; and several were caught under an old turtle's shell with thysanurans.[1] At "19" two or three were observed in a mouse trap with some cheese. At "40" one was caught under an old piece of meat with a small white thysanuran in its mandibles. At "43" one was eating a white-winged dipteron, this spider was running on the wall. At "50" one was seen in its snare eating a small myriopod.

This spider is usually very peaceful. Neither in the caves nor in captivity have I ever seen them to show the least signs of pugnacity when they came in contact with each other. Nevertheless it appears certain that they at times eat each other. A few times their remains have been found in the snares. A few remains were observed in the glass cases in which they were sometimes kept in the laboratory. Quite a number of times remains were found in the collecting vials the next morning when three or four were left in the same vial over night.

When bits of dirt were thrown into the snares, the spiders ran away quickly. Blind beetles were caught and tossed into the snares. At the instant when the beetles struck the snare, the spiders ran and with a jump seized the victim. Neither the web, nor spider, nor both together were strong enough to hold the beetle. Large flies were also thrown into the snare with the same results. The spiders always seized their prey and held on tenaciously until the last second. Small flies and mosquitoes were likewise tossed into the snares. In each case the spider made a quick run and with a jump seized the victims and held on so firmly that both prey and spider were torn loose from the web by picking the prey up with forceps.

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[1] Here as elsewhere used in broad sense to include both *Thysanura* and *Collembola*.

Not one specimen has even been seen drinking water. Since they always live in a saturated atmosphere all the water required is probably absorbed through the skin.

In captivity I have fed *Meta* flies, mosquitoes, gnats and various species of other arachnids smaller than themselves. When two or more of them are placed in the same case the largest invariably devours all the others in a short time.

In the caves *Metas* have been observed at various localities eating gnats, mosquitoes, flies and cave crickets. One was seen eating a small moth and another an old dried myriopod.

Enemies.

As far as my observations go, these spiders have no enemies besides themselves. It is very seldom that one can find the remains of a specimen in the webs and as mentioned above no case of fighting has ever been witnessed. At "63" almost a mile from the entrance the only other insect found was the blind beetle. Doubtless carefully repeated observations at this locality will prove that other insects are also present.

Temperature.

During the last three years a series of temperature records[1] have been taken at "10," or in the "Big Room." For 1907 the temperature is as follows: 11.5° C. for January, February, March, April and May; June 11.7°; July 11.9°; August and September 12.7°; October 12.2°; November 11.9°; and December 11.7°. Since food is more abundant and the three necessary physical conditions are evidently suitable at "2," "9" and "38," we can probably attribute the small number of specimens to the uneven temperature. Again, since food is extremely scarce a half mile from the entrance while the number of specimens is few, perhaps the small number of spiders is due to the scarcity of food. Midway between these two localities food is comparatively plentiful, the temperature is practically even throughout the year, and this combination is probably responsible for the great number of individuals.

[1] Eigenmann, '09.

Specimens have been kept in captivity in the laboratory throughout the year. In cold weather they are less active than in warm and are very fretful when the vials become too warm, and often die.

COURTSHIP.[1]

November 16 two were seen copulating. In order to see them I got too close and my breath irritated the web. This caused them to separate. After an absence of five minutes I returned and found them together again. A second time they were disturbed. Returning after an absence of fifteen minutes, they were found close together. While copulating, they were lying one under the other with anterior and posterior ends reversed and with the ventral parts of their cephalothorax in contact. November 19, a pair was seen pairing. December 17, two were observed copulating, these were both under the snare, and the anterior and posterior ends of the cephalothorax were reversed. The dorsal surface of the cephalothorax of the male was pressed against the corresponding ventral part of the female. The male placed his palp on the epigynum once, this lasted only a few seconds, then they parted. December 14 and 22, two couples were seen copulating. Those on the latter date were first seen at 2:25 P.M. and then disturbed by breath. At 2:27 they were together again after the male had circled once around the female. This pair was on top of the horizontal snare all the time. The male used his palps alternatively three times in three minutes, each time lasted only a few seconds. They were disturbed at 2:30. January 4, two were observed copulating June 7, a male was seen courting a female. Both were under the snare within one and one-half inches of each other. They were first seen at 3:45 P.M. When the male tried to advance toward the female, she caused him to keep his distance, the result of which caused the male to circle completely around her clockwise in six minutes. Most of the time she kept the posterior end of her abdomen toward him, while he had his head facing her all the time. At 4:00 I left them and a half hour later upon my return they were still in the same position. They were placed in a vial, taken to the house and were put in a case. In this case they lived two weeks where they died. This pair like all others when in captivity had no inclination to mate.

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[1] McCook, '93, describes only courtship of outside forms.

Cocoons and Eggs.[1]

One cocoon was made in a glass case October 4. It contained six eggs. Another was made in a case December 11. January 21, one with five eggs was constructed in a vial. Other cocoons were made in vials on the following dates: April 28; May 5, 8, 22, 27 and 31; June 3, 25 and 28; July 8 and 27; August 6, 19 and 21.

On the following dates cocoons with eggs were collected in the caves: October 8, one with two eggs; one with eight eggs, January 20; January 26, one containing eight newly hatched spiders and one with seven eggs; another with seven eggs March 2; March 16, two cocoons, one with eggs and the other with newly hatched; May 24, one with four eggs; July 2, one with four eggs; July 19, at various localities collected seven cocoons, one of which contained eight eggs, and two others each held four young, at various localities in Upper Dalton August 22 collected seven cocoons. Some of these contained young just hatched, and others young ready to leave the cocoons.

The cocoons in the caves are usually constructed in secluded places and are difficult to find unless one examines every little crevice and looks under the ledges of rocks very carefully. Sometimes they are found attached to the underside of rocks lying on the floor, but more often under little ledges of rocks and in the acute angles of small crevices.

In color they are snow white and are disc-like in shape. The flat part of the disc is fastened firmly to the rocks. The average size is 6 mm. in diameter by 3 mm. in depth, although sometimes a cocoon containing the minimum number of eggs is as large as one containing the maximum number. I have never been fortunate enough to witness a female making her cocoon, but on examination, a coocon is composed of a more or less firm and closely woven circular base. The eggs are piled into a heap in the middle of this base and then the convex part is spun over them in such a crude and unsubstantial way that one can generally count the eggs through this covering. In detaching the cocoon from the rock one must use precaution for fear the eggs fall through the covering.

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[1] Montgomery, '06, describes the cocoons and eggs of an allied out-side form.

In number the eggs vary from two to eight with five for an average cocoon. They are transparent whitish in color and are perfect spheres with an average diameter of 0.6 mm. During the embryological stages, they soon take on a yellowish color, become oblong in shape, and the outline of the embryos is discernible through the covering. Some of these embryos assume the shape of the profile of a man's head.

YOUNG.

When hatched they remain for an indefinite period inside the cocoon and when strong enough emerge through a small circular hole.

March 22, three of the seven eggs in a cocoon collected March 2, hatched; April 3, two of these spiderlings were dead, they with the remaining eggs were covered with mold. June 4, two of the four eggs in a cocoon collected May 24, were hatched, one spiderling was dead and the other alive on this date. Neither one had any eyes. July 29, a cocoon collected July 19, was examined and contained three young. Each one was examined both alive and dead. All eyes, except the anterior middle ones, were discernible. Female no. 139 made a cocoon and laid four eggs May 5. On May 23 all four eggs were hatched, but the young were still inside the cocoon. Each spiderling had all eight eyes except the anterior middle ones. The eyes had a uniform diameter of 0.018 mm. While alive under the microscope their little eyes shone like small electric lights. Their mother had no signs of external eyes. Many other newly hatched spiderlings were observed both alive and dead. The anterior middle eyes are never discernible. In some, the other six eyes are present and in others no eyes can be seen. All the other eggs laid in the laboratory failed to hatch. Perhaps this was due to uneven temperature.

The young are much thicker-set than the old. The legs are thick and stubby. The cephalothorax and legs are transparent whitish while the abdomen is light cream in color. The latter has a few longitudinal rows of hairs. The length varies from 0.6 to 0.8 mm.

Moulting.

While in captivity seven individuals moulted, three of which were found dead shortly after the skins were cast off. The deaths were probably partially due to an excess of water in the vials for immediately after the old skins were shed the spiders lay lifeless in the water. The skins were suspended by threads to the upper side of the vials. The moults show that the skin splits on each side of the cephalothorax at the dorsal side of where the legs are attached. Hence, the moulted skin of legs and mouth parts adhere to the ventral half of the moult, and the corneal cuticle belongs to the dorsal half. All the old hairs are shed along with the skin, new ones take the place of the old which causes the color to be perceptibly brighter. The moulted skins of the abdomen were either missing or rolled up into little wads so that one could not tell precisely how they were cast off.

Mortality.

In the caves one never finds dead specimens. In captivity mortality is not great. The most important requirement is to place them in a saturated atmosphere as soon as caught. Two thirds of the specimens were found dead in the collecting vials the following morning when left in the vials without a drop of water over night. It is impossible to keep them long in anything not air-tight, however careful one is to keep them supplied with water. The best device is small vials with air-tight cork stoppers. In such vials they may be kept for months without food. One caught September 10 was placed in a small vial containing two drops of water. On January 20 more water was added which almost drowned the specimen. On January 26 it died. During all this time it had had nothing to eat. Another individual was placed in a small vial January 7, and died April 19, due to lack of moisture or food.

In captivity they quite often die soon after moulting. In vials sunshine kills them in a few minutes. The heat from a student's lamp is also fatal.

Light Experiments.

In the caves one may throw the light from a carbide bicycle lamp on *Troglohyphantes* for a half hour or more without producing any effect. Such is not true with *Meta*. Just as soon as the light strikes their eyes, they run into the dark. If the light is repeatedly thrown on their eyes, they may be turned in any direction and often can be driven into places where the light cannot reach them.

The following apparatus was used in the laboratory: For the adult *Metas*, slender 10-inch bottles; for the medium sized *Metas*, 6-inch test-tubes; for the spiderlings of *Meta* and for *Troglohyphantes*, small 5-inch vials. The closed end and the lower half of each vessel were covered with black carbon paper. The open ends were securely closed with air-tight cork stoppers. One specimen with one or two drops of water were placed in each vessel. In a very short time the water forms a thin film of moisture all over the inner surface of the vessel. This saturated the air in both ends equally. The vessels were then placed on an inclined rack by a south window in order to give each an equal amount of light. Occasionally they were rotated so that the light always fell directly upon the spiders' eyes. At various times the carbon paper was transferred to the cork end, thus throwing the specimen into the light or dark as the case may be. Those that were strongly negatively phototropic never lost much time in finding the dark end, regardless of the number of times the carbon paper was changed. Such individuals often pass into the dark in three minutes. The few that were strongly positively phototropic always changed from the dark end to the light end whenever they were thrown into the dark. Some were thus experimented with for thirty days, but experience taught that their actions were reliable for only the first four or five days. Darkness and cloudy weather had much to do with the final results. Time was counted from the period when first placed in the vessel, and each morning when first observed until 6:00 P.M. each day for four consecutive days. When first placed in the vessels and for a short time after the carbon paper was transferred, they were noticed every few minutes, after that irregularly five times every day.

The adult *Metas* were always in the dark end during clear and cloudy weather, and always in the light end when it was dark. The medium sized *Metas* were always found in the dark end in clear weather, one half the time in the dark end during cloudy weather, and usually in the light end when dark. The spiderlings of *Meta* remained in the dark end one half the time in clear weather, one third the time in the dark end during cloudy weather and most of the time in the light end when dark.

All eight eyes in the *Metas* were present and presumably well developed. The anterior middle ones were generally a little smaller than the others and occasionally an eye was found among the others which was about one half size. In all these experiments forty *Metas* were used.

The following table gives the results of the light experiments for *Troglohyphantes*. On the left is entered the number of the specimen, the locality, the sex, age (whether mature or immature) and the four sets of eyes. AME stands for anterior middle eyes, PME for posterior middle eyes, ASE for anterior side eyes, and PSE for posterior side eyes. All measurements were made with a micrometer slide inside the two inch ocular with two thirds or low objective. When the eyes were scarcely discernible the one-inch ocular was substituted for the two-inch. As a unit of measurement for the eyes one fifty-fifth of a millimeter (0.018 mm.) is employed. The fractional parts of this unit are only approximate. The +'s are used when the eyes are joined together, if separate −'s are employed. P stands for pigment speck. The remainder is self-explanatory.

The thirty examples included in the table were selected, not on a phototropic basis, but to represent the various localities, the age, size, degeneration of the eyes, and coloration. If another table were made from the specimens not included in this one, the results would be similar. If a correction could be made for cloudy weather and for the time occupied in going into the dark, the total per cent. of 49 for those in the dark column would be considerably larger than the total per cent. of 51 for those in the light column. In all these experiments 225 specimens have been used and I am positively certain that the results as given in the table are correct.

Summarizing the following table and other data not included therein we have the following statements:

Twenty-six per cent. of all the individuals examined had no

LIGHT EXPERIMENTS FOR TROGLOHYPHANTES CAVERNICOLA KEY.

No.	Loc.	Sex.	Age.	Size.	AME.	PME.	ASE.	P.S.E.	Color Abd.	Color Ceph.	Light, Hrs. and Min.	Dark, Hrs. and Min.	Light, Per Cent.	Dark, Per Cent.
397	9	♂	Im.	2.2	.5+.5	.7—.7	.7— .7	.7— .7	light	pink	11.40	24.45	38	62
712	9	♀	Mat.	2.8	.5—.5	1—.5	1— 1	1.5—1.5	brown	"	15.20	21.05	42	58
322	10	?	Im.	1.0	0— 0	0— 0	0— 0	0— 0	light	light	10.00	25.15	28	72
324	10	♂	"	2.5	.5+.5	1— 1	0— 0	0— 0	"	pink	15.15	20.00	43	57
327	10	♀	Mat.	2.6	0— 0	0— 0	0— 0	0— 0	brown	"	13.00	22.15	37	63
714	10	♀	Im.	2.7	.5+.5	1— 1	1— 1	1— 1	"	light	8.25	28.00	23	77
325	13	♀	Mat.	2.8	1— 1	2— 2	1.5—1.5	1— 1	"	pink	30.15	5.00	85	15
326	13	♀	"	2.5	1+1	2— 2	1— 1	1— 1	light	"	18.00	17.15	51	49
399	13	♀	"	2.8	.5+.5	.5—.7	1— 1	1— 1	"	"	17.55	18.30	45	55
705	14	♂	"	2.7	.7+.7	1— 1	1— 1	1— 1	"	"	23.10	13.15	63	37
747	14	♀	Im.	2.0	0— 0	2— 2	2— 2	2— 2	"	"	2.45	33.35	8	92
729	14	♂	Mat.	2.8	.7—.7	1— 1	1— 1	1.5—1.5	"	"	22.05	14.15	60	40
741	14	♀	"	3.0	0— 0	0— 1	.7— 1	0— 1	"	"	15.50	20.30	44	56
709	19	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	"	"	16.05	20.20	45	55
731	19	♀	"	3.0	.7— 1	1— 0	1— 1	1.5—1.5	brown	"	30.30	5.50	83	17
735	19	♀	"	3.1	1— 1	1— 1	0— 1	0—1.5	dark	"	30.50	2.30	91	9
736	19	♀	"	3.0	.7— 1	1— 1	0— 1	1.5— 2	light	"	36.20	0.00	100	00
359	38	♀	"	2.8	.5—.5	P— 1	0— 0	0— 1	brown	"	13.05	21.40	38	62
374	38	♀	"	2.9	.5—.5	.7—.7	.7— 0	.7— .7	"	"	29.30	9.00	76	24
356	38	♀	?	?	.5—.5	0—.7	0— 0	0— 0	?	"	28.05	6.40	81	19
355	40	♀	Mat.	2.8	.5— 1	.5—.5	1— 1	1— 0	brown	"	15.10	19.35	43	57
371	40	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	light	"	2.25	32.20	7	93
375	43	♀	Im.	3.0	0— 0	0— 0	0— 0	0— 0	dark	"	16.30	22.00	43	57
753	43	♀	Mat.	3.2	.5— 1	1— 1	.7— .7	0— 0	"	"	33.40	0.00	100	00
755	43	♀	"	2.4	1— 1	1— 1	0— 0	1— 1	"	light	25.45	7.55	76	24
725	46	♀	"	3.0	.7—.7	1— 1	.7— 0	1— 1	brown	pink	12.40	23.00	35	65
726	46	♀	"	2.9	0— 0	1— 1	1— 0	1— 1	black	"	10.25	25.15	29	71
721	50	♀	"	3.2	.7—.7	1— 1	1— 1	1— 1	dark	"	14.55	21.20	41	59
722	50	♂	Im.	2.3	0— 0	0— 0	0— 0	0— 0	light	light	12.05	24.10	33	67
331	57	♂	"	2.0	0— 0	0— 1	0— 0	0— 0	black	pink	17.45	17.15	51	49
										Total	549.25	522.30	51	49

[? Abdomen was lost.]

external eyes. Sometimes the eyes are not in their natural position. Often black pigment specks are found where the eyes are absent. The largest eyes are two fifty-fifths millimeter (0.036 mm.) in diameter, being twice as large as those of the newly hatched, but such individuals are comparatively rare. Hence as a rule, the eyes do not grow larger after birth, while the specimens more than thribble themselves in size. Neither locality nor size of the specimen determines the degree of degeneration in the eyes, or the shade of coloration. Generally, the lighter colored the individual, the more degenerated the eyes, and vice versa. Specimens totally devoid of eyes always stay in the dark more than fifty per cent. of the time; those with one or more eyes may stay either in the light or dark more than fifty per cent. of the time, the per cent. depending on the amount of degeneration.

HUMIDITY.

Apparatus.—Glass tubes with one-half-inch bore and twelve inches long were used. The opening and three inches of one end were covered with black carbon paper. The other end was closed with a cotton cloth. A spider and two drops of water were placed in the light end of each tube and the tubes were placed in the light the same as in the light experiments. When first placed in the tubes the specimens wandered from one end to the other. In just a few moments they ceased their wandering and remained within reach of the drops of water. As it was impossible to watch these experiments all the time, quite frequently the drops of water evaporated before new ones could be added. Sometimes when the tubes became dry, the spiders were found in the dark end, other times in the light end. Under such conditions some were able to live only one or two days, some four or five days while others survived ten days. Out of two dozen individuals used not one at any time was ever found in the dark end when the light end was wet. Each specimen was examined, some had eyes and others were devoid of eyes. Judging from the preceding experiments on light the specimens devoid of eyes should have been found in the dark end at times, providing there was no other factor stronger than negative phototropism. Since these specimens remained near the drops of water all the time instead of going into the dark, we conclude that humidity is a stronger factor than negative phototropism.

The same experiments were prosecuted with the spiderlings and medium sized specimens of *Meta*. At times these were found in the dark end when the light end was wet, therefore probably humidity with them is not greater than negative phototropism.

CHANGE OF HUMIDITY.

Apparatus.—The same tubes as used in the preceding experiments for humidity, also a hygrometer was employed. A specimen was placed in each tube and was observed several times each day. The following results show the relative humidity and the number of hours and minutes various individuals lived.

No. of Specimens.	Relative Humidity.	Hrs.	Min.
4	38—36	3	15
2	70—66—59	5	30
1	38—36—44	8	15
4	63—93	8	15
1	95—82—84—88— 92—78	23	35
1	81—60—76—73—100—75—89	31	45
1	100—65—55—60— 72—66—60	33	45

On various dates at the entrance and at the different localities in the caves the relative humidity was recorded. At the entrance it varied considerably on different days, but in the caves, the hygrometer always stood at 100 (saturation point).

These arachnids always live in a saturated atmosphere and it is impossible for them to survive long outside the caves where the variation in the degree of humidity is great. As a general rule the higher the relative humidity (with but a gradual and small amount of variability), the longer they live. Since the above experiments were prosecuted from May 18 to June 8, when the change in temperature was not such as to materially affect these spiders, we must attribute their deaths to the hygrometric conditions.

SUMMARY.

1. *Troglohyphantes cavernicola* Keys. is found everywhere in these caves, where the three following necessary conditions exist—total darkness, a saturated atmosphere, and a suitable place for the construction of snares.

2. The first pair of legs are used as tactile organs.

3. All small winged insects, thysanurans and small myriopods serve as food. Scarcity of food does not entirely limit their distribution.

4. They have no known enemies other than themselves.

5. While temperature outside the caves does not materially affect the adult spiders themselves, it is probable that to the even temperature at localities between 600 and 1,700 feet from the entrance is due the great number of specimens found at this place.

6. Courtship is similar to that of some outside forms.

7. Cocooning is rudimentary. The eggs are few and comparatively large.

8. The young are white and are thicker set than the adults. Some are hatched with eyes, while others are entirely blind.

9. Moulting is comparatively rare and is often fatal. There are all shades from white to black in coloration.

10. In size the eyes vary from a small pigment speck to 0.036 millimeter in diameter. As a rule, after birth the eyes cease to grow while the specimens more than thribble themselves in size. Twenty-six per cent. of all the individuals are entirely devoid of eyes.

11. The degree of degeneration in the eyes and the shade of coloration are not determined by either locality or size of the specimen.

12. The lighter colored the specimen the more degenerated the eyes.

13. The more degenerated the eyes the greater the negative phototropism, and vice versa.

14. Humidity is a stronger factor than negative photropism in determining the location of specimens in the experimental tubes.

15 Change of relative humidity is fatal in a few hours

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Explanation of Map.

Shawnee Cave (the outlet). Sec. I., No. 1.
Closed chamber caused by collapse of roof at Sec. I., Nos. 2–3.
Cascade. Sec. I., No. 6.
Double passage. Sec. I., Nos. 7–8.
Old cross cave. Sec. I., Nos. 9–10.
New passages. Sec. I., Nos. 1–8 and 11–13.
Opening in roof leading to *upper older* levels of cave. Sec. I., No. 14.
"Big Room." Sec. I., Nos. 15, 16, 17, 18, 19, 20, 21, 22.
"Fallen Rock." Sec. I., No. 31.
Lower Twin Cave. Sec. I., No. 32.
Upper Twin Cave. Sec. I., No. 33.
Roof too low for passage of boat. Sec. I., No. 34.
Deepest water in cave, 10 feet 4 inches. Sec. I., No. 35.
Lower Dalton Cave. Sec. I., No. 36.
Upper Dalton Cave. Sec. I., No. 37.

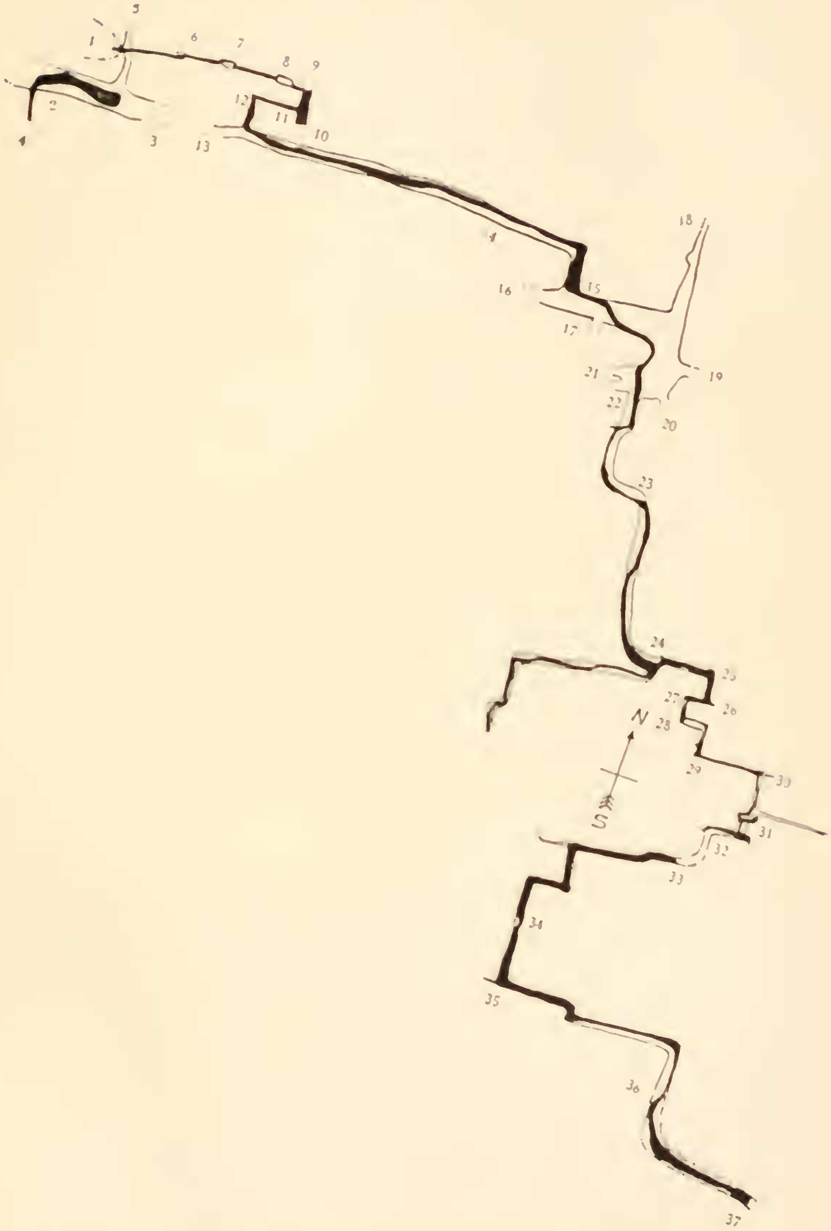

FIG. 1. Map of Shawnee Cave, section 1, from Shawnee to Lower Dalton. Length 4,453 feet. Scale 200 feet to the inch.

EXPLANATION OF MAP.

Upper Dalton Cave. Sec. II., No. 37.
"Cross bedding" in limestone. Sec. II., Nos. 46–47.
"Old passages." Sec. II., Nos. 56–57.
Obstruction past which boat cannot be taken. Sec. II., No. 63.
End of exploration. Sec. II., No. 64.

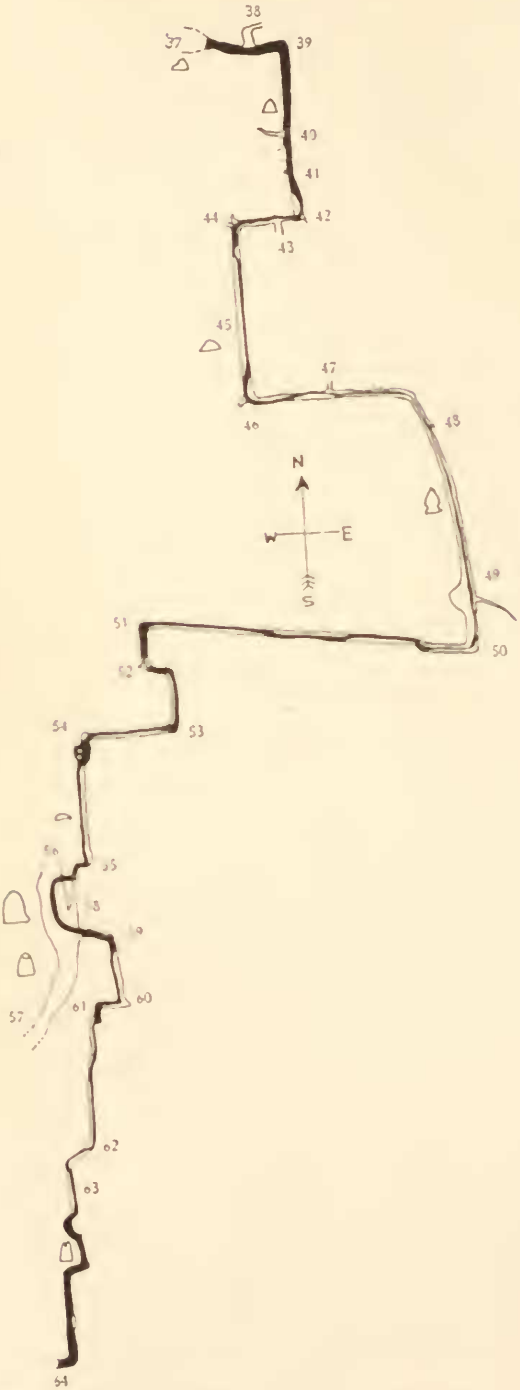

FIG. 2. Map of Shawnee Cave, section 2, from Lower Dalton to unexplored

A NEW SPECIES OF PARAMECIUM (P. MULTIMICRONUCLEATA) EXPERIMENTALLY DETERMINED.[1]

J. H. POWERS AND CLAUDE MITCHELL.

On September 27, 1909, I received from Dr. Powers two sample cultures of Paramecia with the request that I investigate them as to type and purity of culture. To this end I first killed, fixed, mounted and examined 1,000 individuals. They proved to be neither typical *Paramecium caudatum* nor *Paramecium aurelia*, although most of their characters differed but little from these well-known types. Their length ranged between 144 and 288 μ. Their anterior end was a little blunter and the posterior end a little more pointed than even in *P. caudatum*. The cytoplasm was more dense and more opaque. Their chief difference, however, from hitherto described types of Paramecia lay in the matter of the micronucleus, for, instead of the single micronucleus of *P. caudatum* or the two micronuclei of its variety *P. aurelia*, there is a number of very small bodies, evidently micronuclei, ranging in diameter from about .7 to 1.15 μ (Fig. 3). The characteristic position of micronuclei is fully retained, these bodies lying either in slight grooves or in shallow impocketings of the macronucleus. Like the micronuclei of other types, these delicate bodies are always surrounded by a nuclear membrane.

Of the 1,000 individuals examined 875 distinctly showed from two to six of these small micronuclei, 124 showed apparently no micronuclei whatever, while one appeared at first to possess a micronucleus of the type found in *P. caudatum*. This single instance, however, turned out upon careful study to be a case in which a detached fragment of the macronucleus chanced to simulate in size and appearance the regular micronucleus. As to the 124 which appeared without micronuclei, entire degeneration of these bodies may have been possible, but it is more probable that a slight overstain obscured them, especially when lying behind the macronucleus; the same explanation is doubtless true of the individuals in which but two or three micronuclei were found, others existing but in a less visible location.

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[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 101.

Finding thus that the cultures in hand contained nothing but this same type of *Paramecium*, I next proceeded to test the permanence of the type. On October 9, I isolated five individuals, placing each in a clean watch glass containing a definite proportion of sterile and bacterially infected water. These individuals, however, lived but a day. I then again selected five more, varying the proportions of the fluid media. Of these, the two which were placed in water containing the highest percentage of bacteria lived, while the others did not. Three more were started in the same manner as the two successful ones and all these gave living cultures. Of the five living cultures thus obtained two proved much stronger than the others, despite the fact that the conditions were kept as constant as possible in all cases; these two increased rapidly in numbers, while the others increased but little and finally died out after five weeks.

From these two strong cultures, forty individuals were killed, stained and mounted on November 22 and about fifty more on December 17. All of these proved identical in type with the original wild stock. The minute micronuclei were present as before, and again seemed to vary from three to seven in number, which difference depended, in part at least, upon the stain and the transparency of the individual.

Unfortunately during Christmas week extreme cold weather and partial failure of heating plant caused the death of all isolation cultures, the original culture, however, remaining. From this latter, single individuals were again isolated and new cultures started on January 31, the culture media being varied as in previous instances. This resulted again in cultures of varying degrees of strength. One of the best, which I will designate as culture X, was chosen and tested by mounting a number of individuals all of which again proved to be of the multimicronucleate type. This culture X was now accordingly taken as a basis for all further work. From it six cultures were started, the medium being modified in this case by the use of different proportions of agar agar infected with the customary bacteria.

Of these six cultures one, culture Y, was worthy of especial note in that it produced a few conjugants. Early in April this culture became infected with a minute unicellular alga and, possibly as the result of this, the paramecia became more active and increased more rapidly in number. They also ingested the algæ until they became greenish in color. On April 15 six pairs of conjugants appeared. Three of these were killed in about the three-hour stage of conjugation, another in about the seventeenth hour of conjugation, while the other two pairs were isolated, allowed to complete the act of conjugation, and the ex-conjugants used to start new cultures. It was hoped that stronger cultures would thereby be obtained, but this did not follow. They lived and divided slowly for about three weeks only.

The pairs of conjugants which had been killed were stained and mounted in toto, and are of interest as showing, not only that this type of *Paramecium* is capable of conjugation, but something of the nuclear phenomena undergone during the process. In all cases the micronuclei, or at least a part of them, could be made out. In those killed at the three-hour stage (Fig. 1) all were in pairs, indicating no doubt the customary divisions preceding nuclear exchange. In one case three of these pairs were really single nuclei in advanced division. With difficulty the nuclear membrane could be made out, extending, as in the case of the larger dividing micronuclei of *P. caudatum*, between the separating portions of the dividing nucleus. The micronuclei forming the pairs in these three-hour conjugants were smaller than those in non-conjugants. The macronucleus in this stage is still unchanged except that its surface is more or less furrowed.

In the pair of conjugants killed at the seventeen-hour stage the micronuclei are also present, some again in pairs or in division, some single. The macronucleus on the other hand has now broken up into bands and curved segments, simulating a reticulum. This breaking up of the macronucleus at an early stage does not occur with *P. caudatum*, and, in case further study shows it to be habitual with the present type, this will constitute further proof of its independence.

The limited number of conjugants at our disposal and the consequent inability to procure all the stages have prevented our demonstration of the actual nuclear exchange during conjugation, but such exchange is naturally to be inferred from the preparatory division of the micronuclei and from subsequent breaking down of the macronucleus. Every feature of the few conjugating pairs examined indicated normal processes and conditions.

Although the cultures started from the ex-conjugants fared so poorly it is worth noting that shortly after the latter were discovered in culture Y this culture underwent a rapid acceleration in growth and division. This may have been due to undetected cases of conjugation occurring in the culture, or on the other hand it may have been the result of some external stimulus, which itself had caused the conjugation. From this rapidly growing culture two further lots of Paramecia have been mounted, and, as before, all prove to be of the multimacronucleate type. Sections have also been made and stained with iron hæmatoxylin (Fig. 3) from different groups mounted during the year. They have fully borne out the results of the more numerous toto mounts.

All told the cultures have been conducted, the first group for three, and the second for five months. They have not been as strong as could be wished, but the best cultures obtained showed at least no tendency to die out and at the close of the work culture Y was multiplying more rapidly than at any previous time. Had our temperature conditions been more uniform and favorable, we should probably have been able to rear much more copious cultures. The entire uniformity of the type throughout these cultures seems good evidence for its permanence and the probability that it deserves specific rank.

Claude Wm. Mitchell.

I can fully vouch for the methods and results which my student, Mr. Mitchell, has recorded in the first part of this paper. I may speak a few words further as to my own experience with *P. multimicronucleata*. It is not, in the writer's vicinity, a rare or accidental type. Throughout a number of years of work in eastern Nebraska it has been a frequent and troublesome intruder in my *Paramecium* cultures. The most persistent efforts have often failed to procure, from wild stock, pure cultures of *P. caudatum.* A portion, usually the bulk, and frequently the whole, of any culture obtained from pond or river water would turn out to be of this multimicronucleate type.

I did not at first recognize the minute micronuclei. I regarded the individuals, which careful and elaborate technique showed to be lacking in the typical micronuclei of *P. caudatum* and *P. aurelia*, as degenerates in the sense of Maupas' contention. As however the hypothesis of the degeneration of the micronucleus became more and more discredited, I reëxamined mounted slides of these Paramecia under high magnifications, with the result that the minute bodies in question were visible in every case. That this type of *Paramecium* was not related to degeneration was further shown by the fact that many pure cultures, unlike those with which Mr. Mitchell has labored so assiduously, have been vigorous and strong growers. I may further mention the fact that in several very large aquaria supplied with running water and a small amount of fresh meat added occasionally, this type of *Paramecium* apparently maintained itself continuously for several years. As often as the organic matter was supplied the animals would multiply and appear in vast swarms in the corners and protected portions of their space; whenever examined they proved of this type and of this type only.

The existence of an undescribed species of *Paramecium* seems improbable. The protozoa are considered of universal distribution, and *Paramecium* is the most-studied genus in existence. Nevertheless much of the study of mircoörganisms is superficial; many have failed to develop a suitable technique, easy as this is, for the certain demonstration of micronuclei; and as to the hypothesis of universal distribution, it is certainly assumed much further than it is proven. Thus, for the last six years, I have made careful search among cultures derived from very numerous wild stocks, for *Paramecium* of the *aurelia* type, *i. e.*, with the well-known two micronuclei. But, aside from a very few isolated individuals derived experimentally from *P. caudatum*, not a single example has been found.

All in all, it seems that, in the light of Mr. Mitchell's results, the type in question deserves specific rank, although this rank rests upon nuclear differences only. The external characters mentioned by Mr. Mitchell, although holding good for the material investigated by him and for many other lots as well, are not universal, nor have I noted any external character that is. When *P. multimicronucleata* is grown in the same culture with any given strain of *P. caudatum*, the two can usually be readily separated by one or more external characters. Frequently the *P. multimicronucleata* are uniformly larger than the accompanying *P. caudatum* but they do not exceed the known dimensions of the commoner form, and, in some cultures they are uniformly smaller. So with length of groove, form of ends, opaqueness, etc. The most uniform character that I have seen is that the new type is a little more cylindric in form, bulging less at the point of greatest diameter; but *P. caudatum* approaches this form in starved cultures.

The propriety of basing a species upon nuclear characters only depends of course upon their constancy. Calkins has shown that the types of *Paramecium* with single and double micronuclei respectively are not wholly constant, occasional transitions taking place in both directions. He, therefore, pronounced the types varieties. But the conclusion has again been called in question, the infrequency of the transition leading Kofoid to re-interpret the phenomena as instances of mutation.

I have myself been conducting, during a considerable part of the present year, preliminary experiments on *P. caudatum*, subjecting them to different conditions with a view to ascertaining their possibilities of variation. The only striking results have occurred as the consequences of great changes in feeding habit.

P. caudatum is almost exclusively a bacteria feeder. But as Mr. Mitchell has recorded they occasionally deviate to other minute vegetable organisms. This year I have succeeded in inducing a certain percentage of the individuals from a pure culture of very large and strong growing *P. caudatum* to feed on minor animal organisms, first on flagellates (*Chilomonas*) and then, to a considerable extent, upon smaller ciliates. These very striking changes in food habit produced very striking variations in the Paramecia, both nuclear and cytoplasmic. I will not describe these at the present time save in so far as they relate to the present discussion. Many of the nuclear changes were erratic and possibly pathological: Macronuclei greatly enlarged, micronuclei unchanged, or sometimes apparently absent, or again enlarged, even more in proportion than the macronucleus, and sometimes divided.

Among the large mass of such material, stained, mounted and examined, I discovered a very few instances of individuals with two typical micronuclei. In fission these micronuclei divided simultaneously and normally. The number of these individuals was very few, probably not exceeding one to several thousand, but they confirm, to some extent, Calkins's observation that *P. aurelia* may arise from *P. caudatum*.

Among the different types of variants I sought assiduously for examples of *P. multimicronucleata*. But none of the exact type were found. Evidently this type is farther separated from *P. caudatum* than is *P. aurelia*. A considerable number of individuals were found however which showed an approach to *P. multimicronucleata*, in that the micronucleus was divided, usually, again, into but two bodies, perfectly normal in appearance, but much smaller than the typical micronuclei of the genus, though a little larger than those of the new type. This variant was one of the most constant and frequent results of the changed diet. In other characters, however, it did resemble closely *P. multimicronucleata* or, for that matter, any recognized type of the genus. I regard it merely as an instance of the well-known law that a powerful stimulus to variation applied to any species brings out, not only new characters, but characters of existing allied species as well. The phenomena, to the writer, serve to confirm, rather than to refute, the specific independence of the new type. But they are of interest in themselves as showing possible lines of experiment leading to nuclear variation. In the present instance it seemed especially worth while to record them, and indeed this is the chief reason for the entire study, in that *Paramecium* is more and more being made the subject of extensive experimental research. So far, little of this study has had regard to other than external characters, but this admitted limitation must soon be remedied, and to this end it is essential that we know the types of nuclear structure present in the different species or varieties of the genus as well as the lines of variation to which they are subject.

J. H. POWERS.

UNIVERSITY OF NEBRASKA,
July 30, 1910.

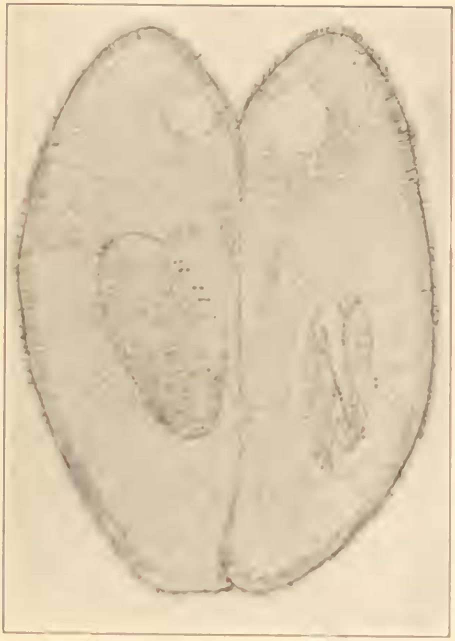
Explanation of Plate I.

Paramecium multimicronucleata.

FIG. 1. Conjugation near the three-hour stage, showing micronuclei in pairs or in division.

FIG. 2. Conjugation at about seventeen hours. Macronucleus already broken into band-like portions. Micronuclei visible in part, in pairs or single.

FIG. 3. Section of typical *P. multimicronucleata*, an unusual number of the micronuclei chancing to lie in one plane.



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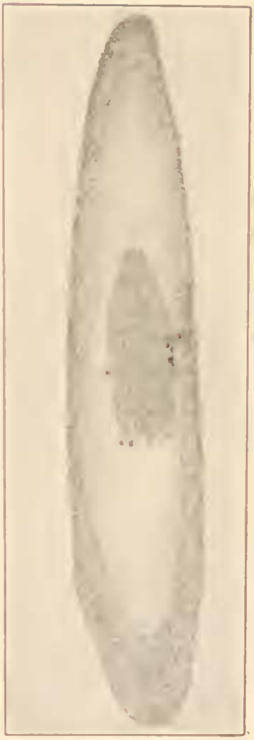

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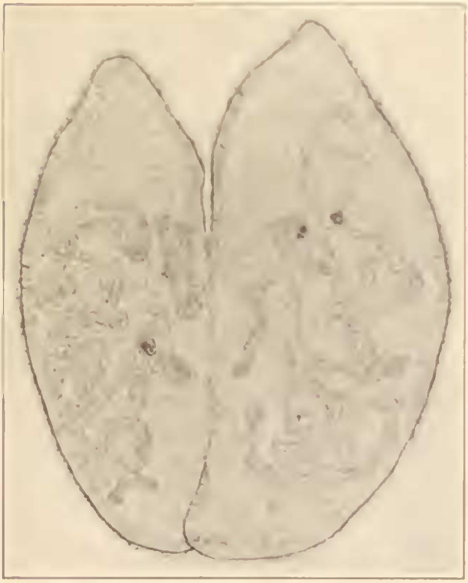

2

THE CENTRAL NERVOUS SYSTEM AS A FACTOR IN THE REGENERATION OF POLYCLAD TURBELLARIA.

C. M. CHILD.

Some years ago L. V. Morgan[1] described a series of experiments concerning the effect of removal of the cephalic ganglia upon the course of regeneration, particularly of the anterior region, in the Californian polyclad, *Leptoplana littoralis*. Her conclusion is that under certain conditions, *i. e.*, particularly in the presence of tissue anterior to the ganglia, regeneration occurs as readily and as completely in the absence of the ganglia as when they are present. At about the same time the results of my own experiments on *Leptoplana tremellaris* appeared.[2] I found that when half or less than half of the ganglionic tissue was removed, regeneration might be as complete and as rapid as when the ganglia were uninjured, but that when more than half of the ganglia was removed, regeneration was not only slower but less complete than when they were uninjured.

I also found, however, that groups of eye spots not infrequently appeared in cases where the ganglia themselves were, so far as could be determined, completely removed, and that the amount of anterior new tissue in all such cases was greater than in those cases where eye spots did not appear, though regeneration was never complete.[3] My general conclusion from these experiments was that the central nervous system in these forms acted as a functional stimulus to the growth of new tissue, increasing both the rapidity and the amount of growth.

The above mentioned experiments of L. V. Morgan seem to indicate that under certain conditions this is not the case. In view of the apparent disagreement between her results and my own further experiment seemed desirable and during the autumn of 1907 and the summer of 1910 I took the opportunity to examine several species of Leptoplanidæ which occur at La Jolla, Calif., with reference to this point. In 1905 I had worked with *L. littoralis* at Pacific Grove and obtained results similar to those described for *L. tremellaris*.

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[1] L. V. Morgan, "Incomplete Anterior Regeneration in the Absence of the Brain in *Leptoplana littoralis*," BIOL. BULL., IX., 3, 1905.

[2] Child, "Studies on Regulation. "V.—The Relation between the Central Nervous System and Regeneration in *Leptoplana*: Posterior Regeneration," *Journ. Exp. Zool.*, I., 3, 1904. "Studies on Regulation, VI.—The Relation, etc.: Anterior and Lateral Regeneration," *Journ. Exp. Zoöl.*, I., 4, 1904.

[3] Child, "Studies on Regulation, VI.," Figs. 15–19, p. 522, Figs. 41–43, p. 526, also p. 529.

My conclusions from this later work are essentially the same as those reached in my earlier paper. Removal of the ganglia with as little of the surrounding tissue as possible always results in decreased rapidity and completeness of regeneration, whatever the method of operation employed. In many cases, however, groups of eye spots appear in the new tissue, even when the ganglia are wholly absent, and in such cases the regeneration is always more rapid and more nearly complete than when the eye spots do not appear.

When the ganglia are removed by a cut from one side of the head, as in some of Morgan's experiments, more new tissue is often formed, or it forms more rapidly, in the deep cleft made by the cuts than on a nearly flat terminal surface. This, however, is not due to any specific effect of the anterior tissue, but is merely a very general characteristic of wound-healing, not only in *Turbellaria*, but in many other forms, and is doubtless due to the fact that nutritive and other conditions for growth are better in such a cleft, where the growing parts are in contact on both sides with other tissue, than on surfaces where such contact exists only on one side.

I believe that the important point in connection with the problem of the influence of the central nervous system on regeneration in these forms lies in the question as to what constitutes the central nervous system. As Morgan states, the cephalic ganglia in the polyclads are enclosed in a definite sheath, but a further point of great importance which she does not consider at all is that the nerve roots contain numerous ganglion cells for a considerable distance from their point of origin in the ganglia. Reference to Lang's monograph of the polyclads[1] is sufficient to establish this point. There is then every reason to believe that the central nervous system comprises, not the ganglia alone, but the ganglia plus the nerve roots for a certain greater or less distance from their origin. Even when the ganglia are completely removed, the capacities of the central nervous system for regeneration and stimulation are not wholly lost, if sufficient portions of the nerve roots near the ganglia remain. In such cases the amount of regeneration is greater than when the nerve roots are also removed, and groups of eye spots may appear. In fact, in one case[1] I observed the regeneration of a small but distinct ganglionic mass after the apparently complete removal of the ganglia. It seems not improbable that if our technique were sufficiently exact to permit removal of the ganglia without injury to the nerve roots except at their origin, the regeneration even of the ganglia themselves, as well as of other parts, might be almost or quite as complete as when the ganglia remain.

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[1] Lang, "Die Polycladen des Golfes von Neapel," *Fauna und Flora des Golfes von Neapel*, XI., Leipzig, 1884.

L. V. Morgan's Fig. B2 of the regenerated anterior end of the nervous system after removal of the ganglia shows only fibrillar structure and she states that only fibrillæ are present in the mass. But when we recall the facts as to the histological structure of the nerve roots it seems extremely improbable that ganglion cells are totally absent from such regenerated masses. In all cases of the kind, which I have observed, some cells as well as the fibrillæ have always been present in the knot of tissue formed by the union of the nerve roots.

The development of eye spots in many of the cases without ganglia described by Morgan is undoubtedly due to regenerative processes in the remaining ganglionic nerve roots. In my own experiments I have found that in all cases, whatever the method of operation, where the ganglia plus a sufficient portion of the nerve roots are removed the regeneration is always slight, eye spots do not develop and the animal never shows any recovery from its sluggish unresponsive condition, *i. e.*, it behaves in all respects like a headless animal. On the other hand, where the roots are largely intact, regeneration is more rapid and proceeds farther, eye spots often appear and the recovery of motor activity and apparent spontaneity frequently occurs to a very marked extent.

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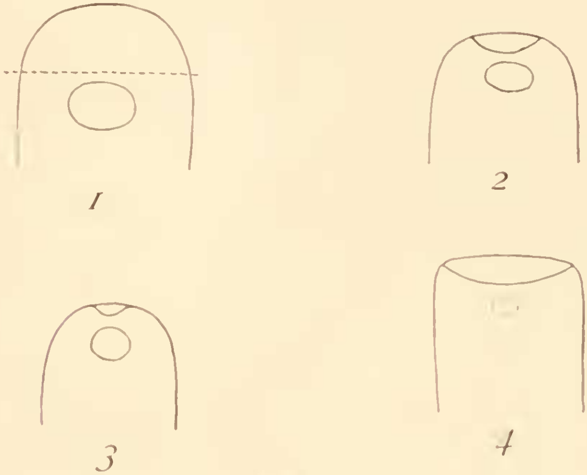
[1] Child, "Studies, etc., VI.," Fig. 43, p. 526.

Since my later experiments confirm in all respects my earlier work, it seems unnecessary to describe them in detail and to figure all the various methods of operation and the results. I can only conclude that the apparent absence of effect of ganglionic removal upon regeneration in certain cases is due to one of two things, viz., failure to remove the ganglia completely, or the presence of the intact nerve roots. Morgan's experiments do not in any way prove that the central nervous system does not exert an influence upon the rapidity and amount, and so far as the sense organs are concerned, upon the character of regeneration in the polyclads.

As regards one point, however, Morgan's results as stated in her paper disagree so completely with my own that some further consideration is necessary. In the concluding paragraph of her paper the statement appears that "regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present." Individuals with the ganglia removed and the end cut off anterior to the ganglionic region regenerated as rapidly and completely as controls with uninjured ganglia and the anterior end cut off at the same level. In these experiments the ganglia were removed by using the cut end of a straw as a punch and after the wound thus made had healed the anterior region of the head was cut off.

I have performed this experiment a large number of times and on various species of Leptoplanidæ and with essentially uniform results, viz., that in all cases where the ganglia were actually completely removed, regeneration was less rapid and less complete than in control experiments with uninjured ganglia. Moreover the larger the portion of the nerve roots removed in addition to the ganglia themselves, the less rapid and less complete the regeneration. The operation is by no means easy to perform successfully and in many cases larger or smaller portions of the ganglia remain: such cases show all gradations from complete regeneration to a condition essentially like the pieces from which the ganglia are totally absent, but they must of course be regarded as unsuccessful experiments for our present purpose.

In my experiments the ganglia were removed with a straw in the manner described above: two weeks later, after the wound had completely healed and the ganglionic region was filled in with new tissue, the anterior end of the head region was removed by a cut as indicated in Fig. 1. At the same time the anterior ends were removed at the same level from another series of individuals with uninjured ganglia.



FIGS. 1-4.

The condition of the animals without ganglia a week after the second operation is indicated in Figs. 2 and 3, while Fig. 4 shows the condition of the controls. The difference is marked and requires no comment. After two weeks more regeneration is almost or quite complete in the controls, while the animals without ganglia remain essentially as before and regeneration never proceeds further in them.

In these experiments great care was used to be certain that the ganglia were entirely removed. In various species the ganglia can be seen quite clearly from the ventral surface and examination from this side after the operation will usually show even rather small pieces of the ganglia if they remain. Morgan does not state how the total absence of the ganglia was determined in her experiments and it seems probable that in cases where the anterior end of the head regenerated as rapidly and as completely in animals supposedly without ganglia as in those with uninjured ganglia some portions of the ganglia remained. It is possible that in some cases where the nerve roots were largely intact regeneration might be almost as rapid and complete as when the ganglia are present, but it is certainly impossible to make an extensive series of operations which are uniform in this respect. Morgan's experiments of this kind included however, only "several" worms.

The only conclusion possible seems to be then that the central nervous system, *i. e.*, the nerve roots near their origin from the ganglia, as well as the ganglia themselves, do affect in marked degree the rapidity and amount of regeneration of the anterior regions and, at least as regards the sense organs, its character as well. Moreover, where the ganglia, or the ganglia together with the nerve roots, are removed the method of operation makes no essential difference in the result. As most experiments, not only on the turbellaria but on other forms, indicate, it is probable that the early stages of the formation of new tissue are largely or wholly independent of the nervous system, but it is difficult to understand how the nervous system of an adult animal could fail to affect the amount and rapidity of growth in a regenerating part composed largely of muscles and sense organs. Absence of such an affect would be in direct opposition to the well established fact of the functional influence of the nervous system on various parts of the organism. The rate of metabolism and consequently the rate of growth—*i. e.*, provided nutritive material is present—in such parts must be in greater or less degree dependent upon nerve stimuli. Such an influence of the nervous system upon growth must, however, be sharply distinguished from the determination of differentiation of parts: the effect of the functional stimulus in the stricter sense is primarily quantitative rather than qualitative, so far as structure is concerned. These points were emphasized in my earlier papers.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
October, 1910.

THE FORMATION OF GERM LAYERS IN ACTINIA BERMUDENSIS VERR.

LEWIS R. CARY,

Princeton University.

While at the Bermuda Biological Station during the summer of 1909 I attempted to secure material for a study of the development of *Actinia bermudensis* Verrill, a viviparous actinian that is abundant between tide marks in the shaded limestone caverns along the shores of the smaller islands. Since this material proved to be lacking in the earliest stages in the development, so that the complete embryology could not be worked out, I have thought it advisable to publish the following account as it covers one of the most unsettled points in the development of anthozoans.

According to McMurrich (1891), the only well authenticated cases among the Cœlenterata in which the endoderm arises by invagination are those of *Nauthisoe* and *Pelagia*, both Scyphomedusæ. Jourdan's (1878) account of the formation of the endoderm in *Actinia æquina* he discredits on the basis of his own observations on *Metridium (marginatum) dianthus*. Kowalevskis's (1873) account of the process in *Adamsia rondeletti*, which in the original was inaccessible alike to Professor McMurrich and myself, he dismisses in the same manner saying that it was probably an error in interpretation.

Appellöf (1900) describes the endoderm formation in two species of actinians: *Urticina (Tealia) crassicornis* and *Actinia æquina*. His observations on the latter species confirm the opinion of McMurrich, namely, that there was no true invagination. In the case of *Urticina*, on the other hand, he describes and figures a true invagination, which from his figures could not be considered as an error of interpretation, since he worked with serial sections.

Faurot (1907) has described a true although rather unusual type of invagination in the development of *Sagartia parasitica* and *Adamsia palliata*.

In *Metridium*, according to McMurrich, the result of segmentation is the formation of a hollow blastula with a considerable cavity. Later the inner ends of the cells are constricted off—by the appearance of vacuoles in the line where the separation is to occur—to form the endoderm. At the time when this process is finished there appears at one pole of the blastula a slight depression which gives the embryo, when it is seen in optical section, an appearance similar to the early stages of a true invagination (Fig. 1).

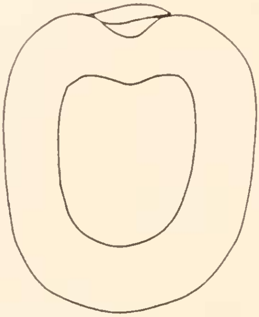


FIG. 1. Pseudogastrulation in *Metridium*. After McMurrich.

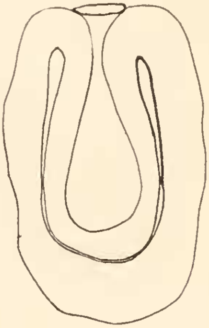


FIG. 2. Later stage in the pseudogastrulation of *Metridium*. After McMurrich.

When the mouth has broken through, the resemblance to an invaginate gastrula is even more complete, so that until such embryos had been seen in sections it would be almost certain to mislead any observer. In reality, however, the two layered condition had been reached before the mouth was formed. This so called "Pseudo-gastrula" McMurrich held to be the true condition in those forms in which invagination had been reported to occur.

All the material of *A. bermudensis* was obtained by slitting the adult individuals longitudinally, and then washing the embryos into a dish of sea water with the stream from a pipette. All stages including the young in which the second series of tentacles was complete, and which were ready to be liberated from the body of the parent were capable of swimming about actively by means of their cilia.

The earliest segmentation stages were never found among the material obtained by washing out the adults, nor did sections of the mesenteries of the adult show any segmenting eggs, so the processes leading up to the formation of the blastula cannot be considered. In the section represented in Fig. 3, the blastula is completely formed. The cells are very numerous and of practically the same height over the entire circumference of the blastula. The interior of the blastula is to a great extent filled with a comparatively thin, lightly staining, plasma-like material, in which there are many yolk granules.

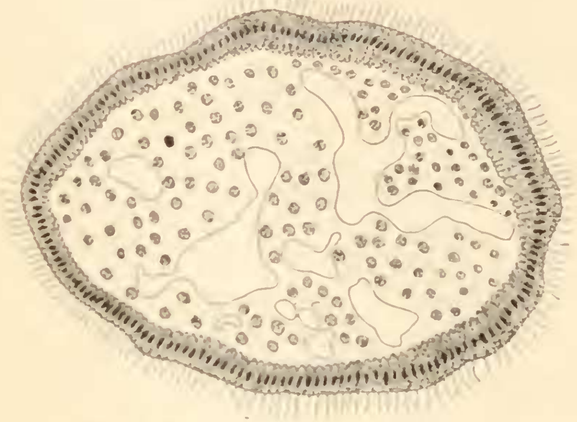


FIG. 3. Early blastula of *A. bermudensis*. Leitz 1/12 and no. 2 (reduced).

The manner in which the separation of the yolk spheres took place during the segmentation can be conjectured only, but, from the appearance of the stage just described it would seem probable that, just as in *Urticina*, there is never an extensive blastula cavity. Instead the yolk material is probably separated from the cytoplasmic portion of the cells in an early stage of segmentation.

In a later stage (Fig. 4) the blastula has become more elongated. The cells have become relatively thinner, and higher, while at one pole there is the first indication of the depression that marks the beginning of the infolding of one portion of the blastula wall to form the endoderm.

Within the interior of the blastula, there has been a marked increase in the relative amount of space unoccupied by nutritive materials. The plasma-like substance has disappeared for a considerable proportion and the yolk spheres have undergone an apparent disintegration. Their outlines are no longer distinct, as in the earlier stages, and in many instances, they can be observed breaking up into rather coarse granules which are being spread out through the surrounding plasma-like material.

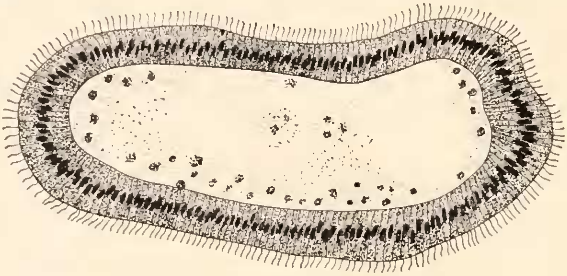


FIG. 4. Blastula of *A. bermudensis* just at the beginning of the invagination.

In the stage shown in Fig. 5 the process of invagination has gone on until the section shows a well-marked early gastrula, and puts beyond any question the type of endoderm formation in this species. The character of the cells in the invaginating area has not as yet undergone any change to distinguish them structurally from those making up the ectoderm.

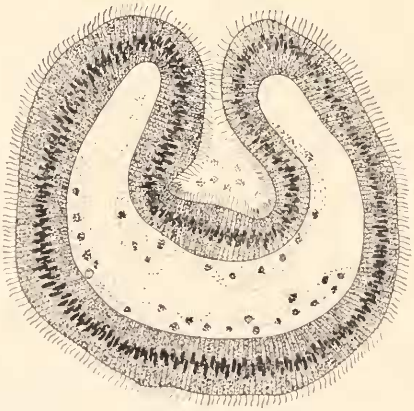


FIG. 5. Invagination of *A. bermudensis*.

The nutritive material within the original blastocœl shows practically the same conditions as noted for the stage last described. Within the gastrula cavity there have appeared some few masses that are apparently composed of the rather coarse granules that come from the breaking up of the yolk spheres.

In the older gastrula, Fig. 6, almost all of the nutritive material has come to lie within the gastrocœl, only a comparatively few of the masses of granules, resulting from the disintegration of the yolk spheres, remaining in the original blastula cavity.

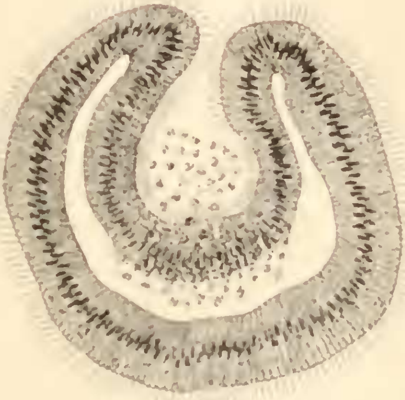


Fig. 6. Later invagination stage. *A. bermudensis.*

Appellof (1900) describes the same sort of migration of the nutritive material during the invagination of *Urticina.* He raises the question as to whether the yolk material seen in the gastrocœl after the invagination is complete is the same as that seen in the blastocœl before invagination, and if so how the transfer could take place. Of the two possibilities in the way of a transfer he mentions: first that the yolk spheres might be absorbed by the cells of the invaginating layer and then be set free to collect in the gastrocœl; and secondly that the yolk elements pass ("sich drangen oder gedrängt werden") between the cells of the invaginating layer. According to his interpretation, his material supports the last mentioned view as many of the cells appear shrunken in diameter while yolk spheres may be seen between such cells separating their lateral walls one from another. He mentions besides that the walls at the inner ends of the cells are often very indistinct during the time when this process is going on, although over the remainder of the gastrula wall there is no appreciable change in the characteristics of the cells.

Appellöf makes no direct mention, although his Fig. 13, Pl. 2, shows, that while the transfer of the nutritive material is taking place the yolk spheres are breaking up rapidly, so that to infer that the spheres pass through the invagination layer in their original condition is unnecessary. In the older gastrulæ of *A. bermudensis* a complete yolk sphere was scarcely ever found in the gastrocœl. While many of the masses of nutritive material still retained their identity and practically their original volume it could be observed in every instance, that the sharp outline was no longer apparent, and usually the granules were separated from one another. It is also noticeable in the section shown in Fig. 6, that nearly all of the yolk material present in the blastocœl is massed about the invaginating cell layer and that the inner ends of these cells are much less sharply defined than they were in the younger stages, Fig. 5, or than they are more laterally in the invaginating area. The central part of the invaginating area is more densely filled with granules than are those parts farther to the sides. The granules in this denser area are also markedly larger than the granules in the cytoplasm of the more laterally placed cells.

It seems, then, beyond question that in *A. bermudensis*, just as in *Urticina crassicornis*, there is an actual passing of the yolk material, in a practically unaltered state, through the layer of invaginating cells to the forming gastrocœl. As the cells of the invaginating layer approach those of the outer gastrula wall all of the yolk passes through so that the two layers come into contact and the supporting layer is secreted between them.

In an older embryo, Fig. 7, in which the formation of the stomodeum has begun, the gastrocœl—gastro-vascular cavity—still contains a considerable amount of the yolk material which now appears as distinct granules. In nearly all instances, however, the granules are arranged in groups which show clearly their origin from an originally more circumscribed mass.

In this last-mentioned stage the cells making up the endoderm have undergone a considerable change so that their histological characteristics are very different from those of the ectoderm cells which have retained very nearly the appearance of the original blastula cells. The endoderm cells have now become much broader in proportion to their height, their cytoplasm is much less dense than formerly, and takes the stains less readily, while the nuclei have become markedly less conspicuous. In some regions, where the body of the embryo is most contracted, especially about the region of the forming stomodeum, the outlines of the endoderm cells are very indistinct, and indeed impossible to make out at the proximal ends of the cells.

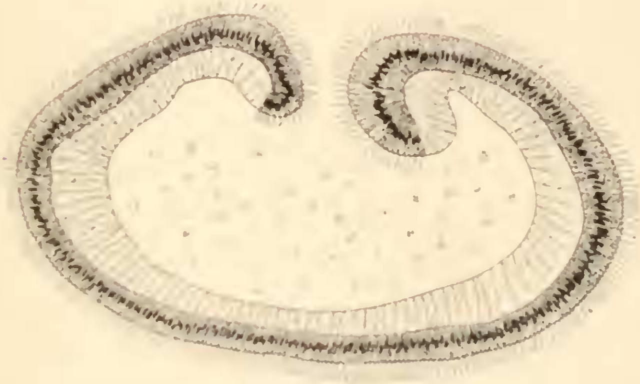


Fig. 7. Young embryo in which the stomodeum is forming. Leitz 7 and 2.

In regard to the interpretation of the optical sections shown in McMurrich's Fig. 10, Pl. XIII., it is interesting to compare my Fig. 6, with the figures of the embryos of *Actinia aquina* given by Appellöf. In the last mentioned form, where the endoderm arises by delamination, there occurs at the time of the breaking through of the mouth opening a decided thinning of both germ layers at the point where the mouth opening will appear. When the mouth has been formed the tissues are very thin all around it, and in a longitudinal section there appears about the mouth this thin area instead of the more thickened area which is found about the gastropore of an invaginate gastrula. So that in some instances at least, McMurrich's criticism of the interpretation of optical sections of whole mounts would not apply.

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